

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

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Date:

15.1.2014

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

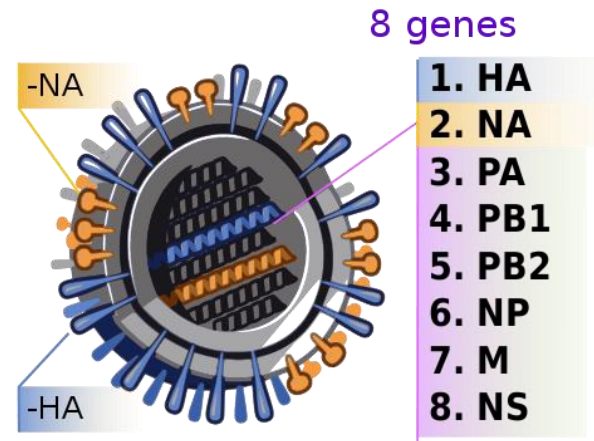
1. Introduction:

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

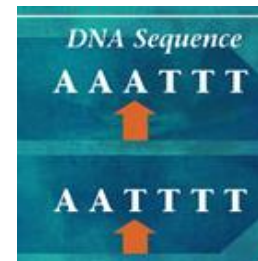
Influenza is considered to be one of the life threatening infectious diseases.

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

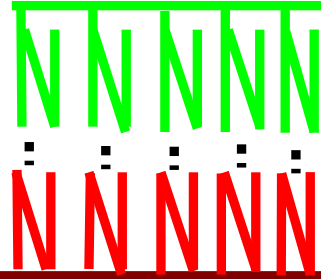


Chip

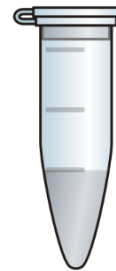
CombiMatrix ElectroSense™: Microarray and hybridization



Surface of chip



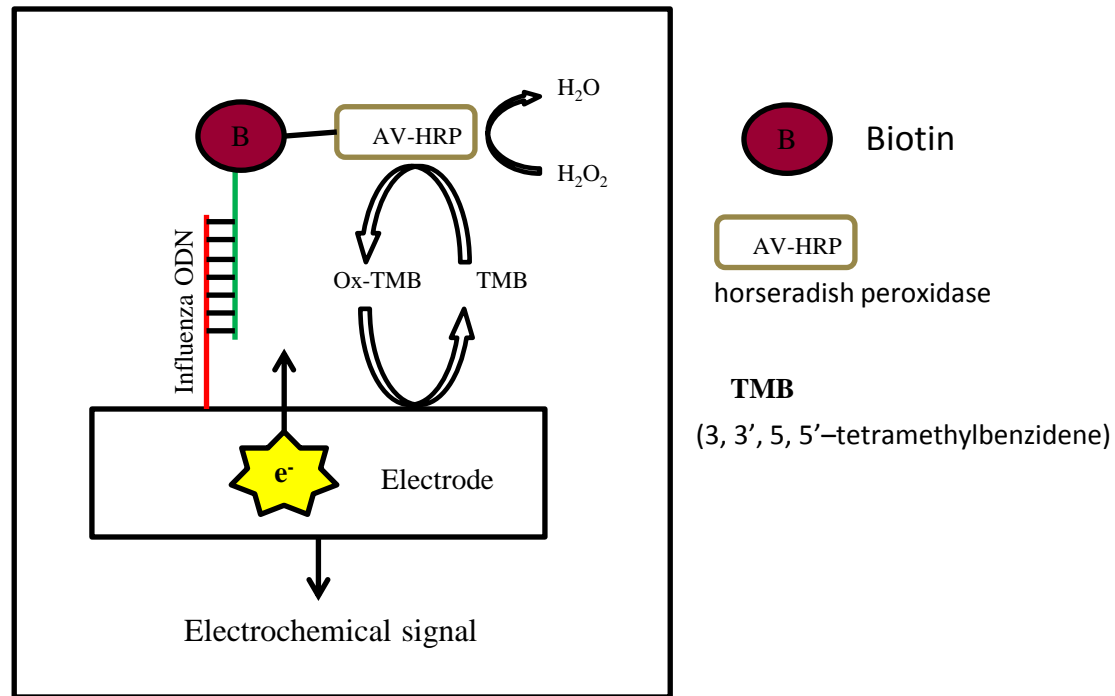
Oligonucleotides probes of influenza viruses inside of the chip



Samples of RNA Influenza viruses



CombiMatrix ElectroSense™: Electrochemical detection



- 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 ElectroSense™ Reader.

**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)

Virus	Gene	GenBank accession number	Sequence
Seasoul A(H2N1) Pseudov: A(H2N1)	nsruncapsidase	CY076797	5'-TCTCTATAATGAAAATDGG-3'
HE22	nsruncapsidase	Q077707	5'-TCCATATGATGAAATDGG-3'
HE22	nsruncapsidase	CY128964	5'-TAGCAATTGC AOGGATCTA-3'
HE22	nsruncapsidase	HE68207.1	5'-TGGATCAAGAGCCCAATTTT-3'
A(H2N2)	nsruncapsidase	Q020952	5'-ACATATGAAAGGCTCTCTT-3'
HE22	nsruncapsidase	IQ433811	5'-GTTRATCATGATTTCAATGG-3'
Seasoul A(H2N1)	nsruncapsidase	CY053217.1	5'-ACCGTTAAAGACAGAAGC-3'
HPAI A(H2N1)	nsruncapsidase	BU26952.1	5'-AAGGAAAGTATTAAATTCAG-3'
HE22	nsruncapsidase	AY344079.1	5'-CCACAAATCTCTGAAAGCT-3'
HE22	nsruncapsidase	NC_004909.1	5'-TAGATGATAGGAGTCCCAAT-3'
Seasoul A(H2N1) Pseudov: A(H2N1)	hemagglutinine	JN017181.1	5'-AGAGAAAGAAATAGATGGGTA-3'
HE22	hemagglutinine	FM25498.1	5'-ACCACAAAGCTCAGCAAAATC-3'
HE22	hemagglutinine	CY128918.1	5'-TGRTGGGACA TA CAATCTD-3'
HE22	hemagglutinine	HE68205.1	5'-ATCAACACTRAATCAAGAGGT-3'
A(H2N2)	hemagglutinine	FM25465.1	5'-TTTTTGTGACACCAACCAAG-3'
HE22	hemagglutinine	IQ433819.1	5'-GACCTTAAAGCACTCAAGC-3'
Seasoul A(H2N1)	hemagglutinine	CY053215.1	5'-OOGAATACACCACCTAAT-3'
HPAI A(H2N1)	hemagglutinine	BU26951.1	5'-CTCCAGAAATCTCCATCAAAA-3'
HE22	hemagglutinine	AY338499.1	5'-GAGACCGGTGGACGCAAAA-3'
HE22	hemagglutinine	NC_004908.1	5'-GTAOTCAATGTCAGACTGA-3'
Seasoul A(H2N1) Pseudov: A(H2N1)	nucleocapsid protein	CY087021.1	5'-ATAGACGGAAATGGATGAG-3'
HE22	nucleocapsid protein	FM25393.1	5'-GAGAGCTTATGCAATATGCG-3'
HE22	nucleocapsid protein	CY128971.1	5'-AGAGAGCTAATGGAAAGTGG-3'
HE22	nucleocapsid protein	HE68206.1	5'-AATGGAAGAGACCAAGCC-3'
A(H2N2)	nucleocapsid protein	CY113840.2	5'-GATTAATGACCGGCTGAT-3'
HE22	nucleocapsid protein	IQ433820.1	5'-CTTCACTTATAGACCAAA-3'
Seasoul A(H2N1)	nucleocapsid protein	CY053218.1	5'-GCGTAAATGGAAGAGAC-3'
HPAI A(H2N1)	nucleocapsid protein	BU26953.1	5'-ACATATTCAGAACAGAGAG-3'
HE22	nucleocapsid protein	AY344241.1	5'-CTCATGCGGATGATAAGCC-3'
HE22	nucleocapsid protein	AF255743.1	5'-GAAAGGTTGATCCAGAAC-3'
Seasoul A(H2N1) Pseudov: A(H2N1)	matrix protein 1	CY087023.1	5'-ACTATCAAGAAAGCTCAAAAG-3'
HE22	matrix protein 1	FM25394.1	5'-ATGATAAGAGATGATGATCTC-3'
HE22	matrix protein 1	CY128951.1	5'-TTAGAGGGAATAACATTC-3'
HE22	matrix protein 1	HE68208.1	5'-CTTTTCTCAAAAATGCGCTAA-3'
A(H2N2)	matrix protein 1	CY113832.1	5'-AAGACTGTGACAGATGCTCT-3'
HE22	matrix protein 1	IQ433821.1	5'-TACAGGAAAGCTTAAAGGGA-3'
Seasoul A(H2N1)	matrix protein 1	CY053216.1	5'-TCTGTCTACCTCTACTA A-3'
HPAI A(H2N1)	matrix protein 1	BU26954.1	5'-ACTGTCCGCTGAGGTTT-3'
HE22	matrix protein 1	GU053111.1	5'-CCCACTAATCAGGCAATAA-3'
HE22	matrix protein 1	AF178646.1	5'-CAAGAAAGCTGAGAGGAAA-3'
Seasoul A(H2N1) Pseudov: A(H2N1)	matrix protein 2	CY087025.1	5'-TCCCTTAAAATACGTTTAAA-3'
HE22	matrix protein 2	FM25395.1	5'-TCTATGAGCTTCTGGACCT-3'
HE22	matrix protein 2	CY128951.1	5'-AACCGCTCTGAAAAGAGGG-3'
HE22	matrix protein 2	HE68209.1	5'-ATCA TGGGA TCTTGCATTT-3'
A(H2N2)	matrix protein 2	CY113832.2	5'-TTTAAA CAGGCTGTBAAGAA-3'
HE22	matrix protein 2	IQ433821.1	5'-GCCTTAAATACGGTTTGA-3'
Seasoul A(H2N1)	matrix protein 2	CY053216.1	5'-TATTA TGGGATCTTCACT-3'
HPAI A(H2N1)	matrix protein 2	BU26954.1	5'-TCCCTTAAAATACGTTTAAA-3'
HE22	matrix protein 2	GU053111.1	5'-CCCTTAAAATACGTTTAAA-3'
HE22	matrix protein 2	AF178646.1	5'-CTTAAAATGCTATTAATGCT-3'
Seasoul A(H2N1) Pseudov: A(H2N1)	polymerase PB1	CY129673.1	5'-AAGA TDTATGACCACTCCCA-3'
HE22	polymerase PB1	HQ246702.2	5'-ATGTGATAA AATCAAAATC-3'
HE22	polymerase PB1	CY129600.1	5'-TGGATGGAATCAAAAATCT-3'
HE22	polymerase PB1	HE68203.1	5'-GGATAAAGAGGAAATGGAAAT-3'
A(H2N2)	polymerase PB1	CY113871.2	5'-TGAATGTTGCTGCAAGAGAAA-3'
HE22	polymerase PB1	IQ433877.1	5'-GATGACTATTCTACAAGACA-3'
Seasoul A(H2N1)	polymerase PB1	JP758919.1	5'-AGCAATGAGCACTBAACATA-3'
HPAI A(H2N1)	polymerase PB1	AY344083.1	5'-AGAAAATGCTACACAAAGAA-3'
HE22	polymerase PB1	AY344083.1	5'-AATCTGGGAGACAACACA-3'
HE22	polymerase PB1	NC_004911.1	5'-ASTTAGAGGCAATGACTGCG-3'
Seasoul A(H2N1) Pseudov: A(H2N1)	polymerase PA	CY129671.1	5'-AAACAGCAATTAATCACTAA-3'
HE22	polymerase PA	IQ433878.1	5'-ATTCATGGAAGAGAGAGTGA-3'
HE22	polymerase PA	CY129623.1	5'-AGAACACTCTTGGATGGAA-3'
HE22	polymerase PA	HE68204.1	5'-GAAAGCCCAACAAGATTAAT-3'
A(H2N2)	polymerase PA	CY113858.2A	5'-CAAGACCACATGATGACTGA-3'
HE22	polymerase PA	IQ433878.1	5'-AAGCAGAGAGCAATTAAG-3'
Seasoul A(H2N1)	polymerase PA	CY053219.1	5'-TCAATGAGGCAAGCTT-3'
HPAI A(H2N1)	polymerase PA	BU26956.1	5'-GCGCAAGCTTCTAAAATG-3'
HE22	polymerase PA	AY342418.1	5'-ACAACCAACCGCCCTCT-3'
HE22	polymerase PA	NC_004912.1	5'-CTTAAAATTAACATTAAGGAC-3'
Seasoul A(H2N1) Pseudov: A(H2N1)	nuclear export protein (nep)	CY129670.1	5'-CATATGAGATGATGACACAA-3'
HE22	nuclear export protein (nep)	HQ240282.2	5'-TGGTAAATTAAGAAATCCCG-3'
HE22	nuclear export protein (nep)	CY128981.1	5'-ACGGAATATGGGAGAACAA-3'
HE22	nuclear export protein (nep)	HE68209.1	5'-TDTATGAGAGTDCGACATTA-3'
A(H2N2)	nuclear export protein (nep)	CY113861.1	5'-AGCAAGTATGCTGTA AATG-3'
HE22	nuclear export protein (nep)	IQ433883.1	5'-TGGCTATTAAGAAAGTCC-3'
Seasoul A(H2N1)	nuclear export protein (nep)	CY053219.1	5'-ACCTCCACTCCCTCAAAA-3'
HPAI A(H2N1)	nuclear export protein (nep)	CY086818.1	5'-GCTTATTAAGAGATGCGAG-3'
HE22	nuclear export protein (nep)	GU053125.1	5'-AAGTCCGACATAGTTGAA-3'
HE22	nuclear export protein (nep)	FP792888.1	5'-TGGCTATTAAGAAAGTCC-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 CustomArray™ 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.

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2. Material and method:

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

Virus	Genes	GenBank accession number	Oligonucleotide probes with one mutation
Seasonal A(H1N1)	neuraminidase	CY076797	5'-TCCCTAAATAAAAATTAGG-3'
Pandemic A(H1N1)	neuraminidase	GQ37078	5'-TCCCTAAAAGGTAATAGG-3'
H2N2	neuraminidase	CY125896.1	5'-TAGCAAAATCGAGGATCTA-3'
H2N8	neuraminidase	BM589207.1	5'-TGGATCAAAAAGCCARGTTC-3'
A(H2N2)	neuraminidase	GQ293982	5'-ACACAAAAGTCTCTCT-3'
H1N8	neuraminidase	IQ433881.1	5'-GTTGAAAACAGT TCAATGG-3'
seasonal A(H5N1)	neuraminidase	CY053525.1	5'-ACCGTTAAAAACGAAAGCC-3'
HPAI A(H5N1)	neuraminidase	EL26984.1	5'-AGGAGAAAATGTGTTAATAG-3'
H7N7	neuraminidase	AY340079.1	5'-CCAGAAAATCTGGAGACT-3'
H9N2	neuraminidase	NC_004909.1	5'-TACATGAAA GGAGLUCCAT-3'
Seasonal A(H1N1)	hemagglutinine	JN017181.1	5'-AGAGAAAATGTGTTGAGGTA-3'
Pandemic A(H1N1)	hemagglutinine	JX625498.1	5'-ACCCTAAAACCTAGCAATC-3'
H2N2	hemagglutinine	CY125918.1	5'-TGTGGGAAATACAAATGCTG-3'
H2N8	hemagglutinine	BM589205.1	5'-ATCATCACAAAATCATGAGGTC-3'
A(H2N2)	hemagglutinine	BM584665.1	5'-TTTTTGTGGAAGCGCAAGG-3'
H1N8	hemagglutinine	IQ433879.1	5'-GACCTTAAAAACACTCAAGC-3'
seasonal A(H5N1)	hemagglutinine	CY053525.1	5'-GGGGAATAAACACCTTAA T-3'
HPAI A(H5N1)	hemagglutinine	EL26984.1	5'-CTCCAGAAAATGCAATGAAA-3'
H7N7	hemagglutinine	AY338459.1	5'-GAAAACGGTGGAGCAAAAAA-3'
H9N2	hemagglutinine	NC_004908.1	5'-GTAGTCAAGATCAGACTGA-3'
Seasonal A(H1N1)	matrix protein	CY087027.1	5'-ATAGCAAAAATGGAATGAG-3'
Pandemic A(H1N1)	matrix protein	JX625388.1	5'-GACGACTAAACGAGATAGC-3'
H2N2	matrix protein	CY125897.1	5'-AGAGGAAAATG GAAAGTGGG-3'
H2N8	matrix protein	BM589206.1	5'-AATGGAGAAAACCGGAGC-3'
A(H2N2)	matrix protein	CY113840.2	5'-GATTATAAAGGGGCGTGTAT-3'
H1N8	matrix protein	IQ433880.1	5'-CTTCAGCTTATCAAAACAAA-3'
seasonal A(H5N1)	matrix protein	CY053526.1	5'-CCCTAAAATGGAGAGAGCC-3'
HPAI A(H5N1)	matrix protein	EL26984.1	5'-ACATCTACGAAAACGGAGGC-3'
H7N7	matrix protein	AY342425.1	5'-CTAATTCGGATGAAAAGGC-3'
H9N2	matrix protein	AF287524.1	5'-GGGAAAGTTCATCAAGAA-3'
Seasonal A(H1N1)	matrix protein 1	CY087025.1	5'-CACTATCAAAAAGCTTCAAAAG-3'
Pandemic A(H1N1)	matrix protein 1	JX625390.1	5'-ATGAAAAGAGATGGCTG-3'
H2N2	matrix protein 1	CY125895.1	5'-TTTAAAAGGAGATACATTC-3'
H2N8	matrix protein 1	BM589208.1	5'-CTTGTCCAAAATGCTTAA-3'
A(H2N2)	matrix protein 1	CY113838.2	5'-ACATATGAAAAGATGTC-3'
H1N8	matrix protein 1	IQ433882.1	5'-TACAGGAAGCTAAAAGGGA-3'
seasonal A(H5N1)	matrix protein 1	CY053526.1	5'-TCTGTGCACCTTACTA A-3'
HPAI A(H5N1)	matrix protein 1	EL26984.1	5'-ACTCTAGGATCAAGTTC-3'
H7N7	matrix protein 1	GU053111.1	5'-CCCACTAAAACAGGATGAA-3'
H9N2	matrix protein 1	AF278656.1	5'-AAAAGAGG TGAAGGGAAG-3'
Seasonal A(H1N1)	matrix protein 2	CY087025.1	5'-TCCCTTAAAAGGGT TAA-3'
Pandemic A(H1N1)	matrix protein 2	JX625390.1	5'-TCATTGGGACTTGCATC-3'
H2N2	matrix protein 2	CY125895.1	5'-AACACGGTCTGAAAAGGG-3'
H2N8	matrix protein 2	BM589208.1	5'-ATATTGGGACTTGCATTC-3'
A(H2N2)	matrix protein 2	CY113838.2	5'-TTCAAACACGGTCTGAAAAG-3'
H1N8	matrix protein 2	IQ433882.1	5'-GCCCTAAAACGGTTTGA-3'
seasonal A(H5N1)	matrix protein 2	CY053526.1	5'-TATCATGGGAACTTGCATC-3'
HPAI A(H5N1)	matrix protein 2	EL26984.1	5'-TGGCTTAAAACGGTTTGA-3'
H7N7	matrix protein 2	GU053111.1	5'-CCGCTTAAAACGGTTTGA-3'
H9N2	matrix protein 2	AF278656.1	5'-CTTAAAAGCTTATTCATC-3'
Seasonal A(H1N1)	polymerase PBI	CY125973.1	5'-AAAATCATGACCACTCCCA-3'
Pandemic A(H1N1)	polymerase PBI	IQ240702.2	5'-AGTGAATGAAAACAAATC-3'
H2N2	polymerase PBI	CY125900.1	5'-TGGAATGAGATAAAAATCT-3'
H2N8	polymerase PBI	BM589203.1	5'-GGATAAGAGAAAAGGAAT-3'
A(H2N2)	polymerase PBI	CY112971.2	5'-TGAATCTGGGCAAAAAA-3'
H1N8	polymerase PBI	IQ433877.1	5'-GATGACTAATCAAAAGACA-3'
seasonal A(H5N1)	polymerase PBI	JP758819.1	5'-AGGATTCAGACTGAAAATA-3'
HPAI A(H5N1)	polymerase PBI	EL26987.1	5'-AGAGAAAGTATACAAAGAA-3'
H7N7	polymerase PBI	AY340083.1	5'-AATATCGGAGAAACACA-3'
H9N2	polymerase PBI	NC_000911.1	5'-AGTAAAAGTATGAAGTACC-3'
Seasonal A(H1N1)	polymerase PA	CY125971.1	5'-AAAACAGATACTCCATCA-3'
Pandemic A(H1N1)	polymerase PA	IQ433878.1	5'-ATTCATGGAAAGGATGG-3'
H2N2	polymerase PA	CY125923.1	5'-AGAAAATCTTTTGTGAGAA-3'
H2N8	polymerase PA	BM589204.1	5'-AGAAAGCTAAAAGATAATCT-3'
A(H2N2)	polymerase PA	CY113838.2	5'-CCAGCCACTGGATCAAAAT-3'
H1N8	polymerase PA	IQ433878.1	5'-AGGGCAAAAGACAATGAAG-3'
seasonal A(H5N1)	polymerase PA	CY053530.1	5'-TCCATGAGGAGCAACTT T-3'
HPAI A(H5N1)	polymerase PA	EL26986.1	5'-GGGCAAGTTCCTCAAAAG-3'
H7N7	polymerase PA	AY342418.1	5'-ACAAAACCGCCCTCT-3'
H9N2	polymerase PA	NC_000912.1	5'-CTGAAAATGATATGGAGAC-3'
Seasonal A(H1N1)	nuclear export protein (nep)	CY125970.1	5'-CATTGAAAAAGTGGAGCA-3'
Pandemic A(H1N1)	nuclear export protein (nep)	IQ240288.2	5'-TGGTAAATGAAAATAGGG-3'
H2N2	nuclear export protein (nep)	CY125898.1	5'-ACCGGAAAAGGCGAGACA-3'
H2N8	nuclear export protein (nep)	BM589209.1	5'-TGGTGGAGAAATACGACATA-3'
A(H2N2)	nuclear export protein (nep)	CY103967.1	5'-AGCAACAGTTGATAAAAGT-3'
H1N8	nuclear export protein (nep)	IQ433883.1	5'-TGGCTGATGAAAAGTGG-3'
seasonal A(H5N1)	nuclear export protein (nep)	CY053529.1	5'-ACCTTCACCTCCCTAAAA-3'
HPAI A(H5N1)	nuclear export protein (nep)	CY096181.1	5'-GCTGATGAAAAGTATGACA-3'
H7N7	nuclear export protein (nep)	GU053125.1	5'-AAGTGGCAAAAGGTGAGG-3'
H9N2	nuclear export protein (nep)	EP792288.1	5'-TGGCTGATGAAAAGTGG-3'



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'-TCCTCATAATGAAAAATTGGG-3'	5'-ACTGCAGCGTAGACGTTTT-3'
M	5'-TCCTCATAATAAAAAATTGGG-3'	5'-ACTGCAGCGTAAACGTTTT-3'
MM	5'-TCCTCATAAAAAAAATTGGG-3'	5'-ACTGCAGCGAAAAACGTTTT-3'
MMM	5'-TCCTCATAAAAAAAATGGG-3'	5'-ACTGCAGCGAAAAAGTTTT-3'
MMMM	5'-TCCTCATAAAAAAAGGG-3'	5'-ACTGCAGCGAAAAATTTT-3'
MMMMM	5'-TCCTCAAAAAAAGGG-3'	5'-ACTGCAGCAAAAAATTTT-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'-TTAAAGGGAGAAACATTC-3'	5'-TCGCTTTAAAAAGGTTTGAA-3'
MMM	5'-TTAAAGGGAGAAAAATTC-3'	5'-TCGCTTAAAAAAGGTTTGAA-3'
MMMM	5'-TTAAAGGGGAAAAAATTC-3'	5'-TCGCTTAAAAAAGTTTGAA-3'
MMMMM	5'-TTAAAGAGAAAAAATTC-3'	5'-TCGCTTAAAAAATTTGAA-3'
	A(H3N2) Hemagglutinine	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'-TTTTGTTGAAACAGCAAAG-3'	5'-AAAAGATGACCAACTCCCA-3'
MMM	5'-TTTTGTTGAAACAGCAAAA-3'	5'-AAAAATGACCAACTCCCA-3'
MMMM	5'-TTTTGTTGAAAAGCAAAA-3'	5'-AAAAAAGACCAACTCCCA-3'
MMMMM	5'-TTTTGTTGAAAAGAAAA-3'	5'-AAAAAACCACCAACTCCCA-3'
	HPAI A(H5N1) nucleocapsid	H2N2 polymerase PA
	Sequence of gen 28	Sequence of gen 63
W/O	5'-ACATATCAGAGAACGAGAGC-3'	5'-AGAACATCTTTGGATGGAA-3'
M	5'-ACATATCAGAAAACGAGAGC-3'	5'-AGAAAATCTTTGGATGGAA-3'
MM	5'-AAATATCAGAAAACGAGAGC-3'	5'-AAAAATCTTTGGATGGAA-3'
MMM	5'-AAAATCAGAAAACGAGAGC-3'	5'-AAAAATCTTTGGATGGAA-3'
MMMM	5'-AAAAACAGAAAACGAGAGC-3'	5'-AAAAATCTTTGGATGAAA-3'
MMMMM	5'-AAAAAAGAAAACGAGAGC-3'	5'-AAAAATCTTTGGATAAAA-3'

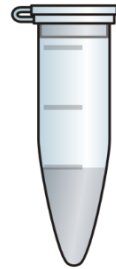
Other part of array have probes specific sequences with one, two, three and four mutations to influenza subtypes



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) Hemagglutinine (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.

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

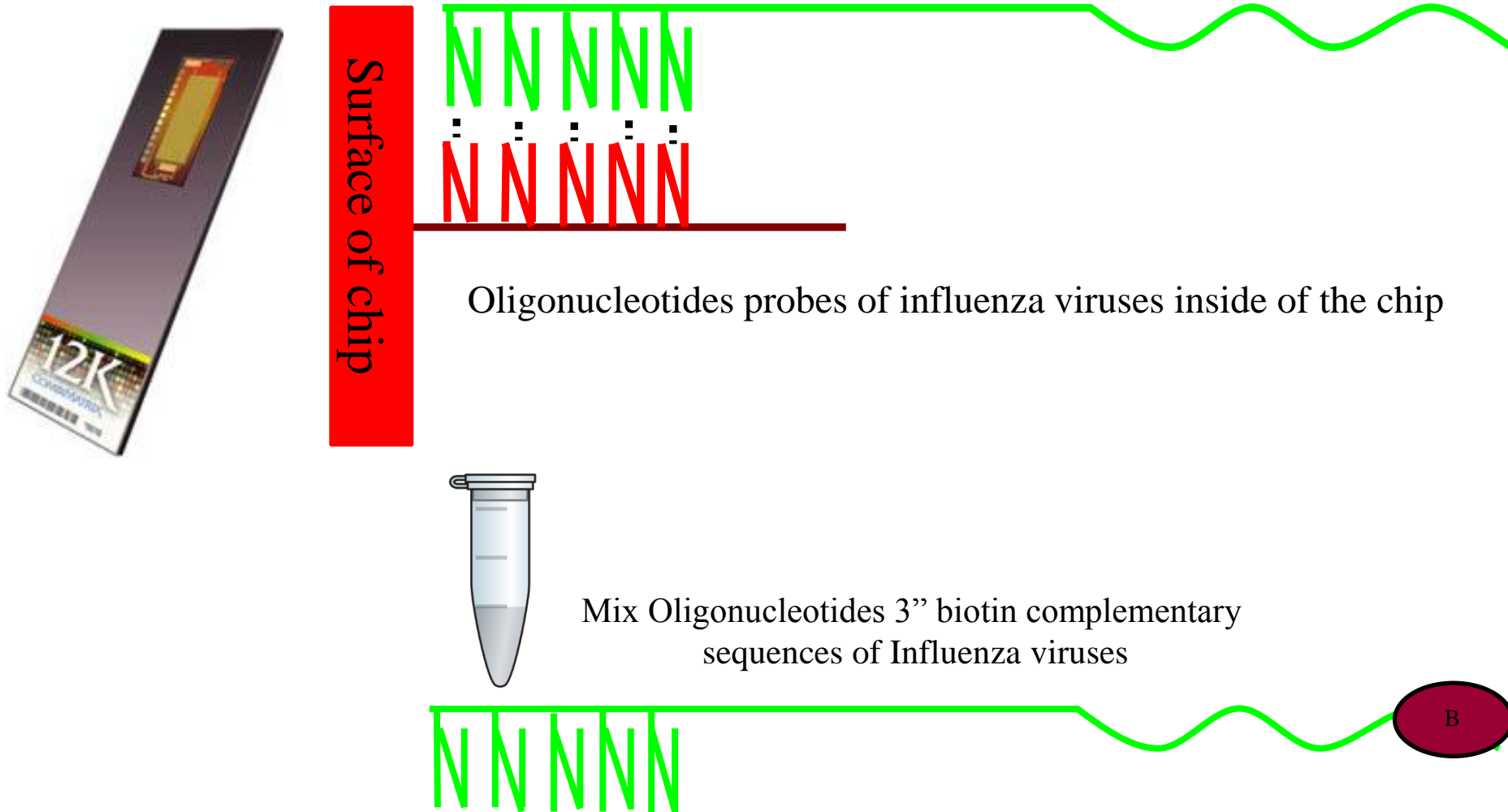


Mix Oligonucleotides 3' biotin complementary sequences of Influenza viruses

Virus	Gene	GenBank accession number	Sequence
Seasonal A(H2N1)	HA	CY18797	5'-TCCTCAATATGAAAATTGGG-3'
Denkerm A(H2N1)	HA	HQ17078	5'-TCCTCAATGTTGTAATTAAGG-3'
H2N2	HA	CY12896.1	5'-TAACAATTCAGGGGATCCTA-3'
H2N8	HA	HM89207.1	5'-TGGATCAAGAGGCCATCTTT-3'
A(H2N2)	HA	CC02019.2	5'-AGACATAGGTTCTCTTTC-3'
H2N8	HA	HQ43881.1	5'-GTTBAATCACTTCAATGCG-3'
Seasonal A(H2N1)	HA	CY15327.1	5'-ACGCTTAAAGACAGAAAGC-C-3'
HPAI A(H2N1)	HA	HU24990.1	5'-AGGAAATGATGTTCAATCA-3'
H7N7	HA	AY340079.1	5'-CCAGAAATCTCTGAGGACT-3'
H5N2	HA	NC_048059	5'-TAGATATATAGAAATCCCACT-3'
Seasonal A(H2N1)	HA	FJ017181.1	5'-AGAGATGAAATGATGAGTTG-3'
Denkerm A(H2N1)	HA	JF825498.1	5'-ACCCAAAGCTAGCAATC-3'
H2N2	HA	CY12918.1	5'-TGTGTGATGATGATGATG-3'
H2N8	HA	HM89206.1	5'-ATCAACGCTATGATGAGGTTG-3'
A(H2N2)	HA	JF844665.1	5'-TTTGTGTAACCCAGCAAG-3'
H2N8	HA	HQ43879.1	5'-GACCTTAAAGGACTCAAGC-3'
Seasonal A(H2N1)	HA	CY15325.1	5'-CGGATGACAGCCCTTAAT-3'
HPAI A(H2N1)	HA	EU24991.1	5'-GTCCAGAAATGATGATGAA-3'
H7N7	HA	AY338459.1	5'-GAAACCGTGAACGAAACAA-3'
H5N2	HA	NC_048068.1	5'-GTGATGATGATGATGATG-3'
Seasonal A(H2N1)	NA	CY18727.1	5'-ATAGACCGAAAATGATGAG-3'
Denkerm A(H2N1)	NA	JF825188.1	5'-GAGGACTAATTCAGAAATGC-3'
H2N2	NA	CY12897.1	5'-AGAGAGTGAATGAAATGTTG-3'
H2N8	NA	HM89206.1	5'-AAATGGAAGAGCCCAACC-3'
A(H2N2)	NA	CY11840.2	5'-GATTAATGAAAGCCGTTTAT-3'
H2N8	NA	HQ43880.1	5'-CTCACTGCTTATGAGCAAA-3'
Seasonal A(H2N1)	NA	CY15328.1	5'-CCGATAATGAGAAAGCC-3'
HPAI A(H2N1)	NA	EU24995.1	5'-ACATCAAGGAAAGCAAGC-3'
H7N7	NA	AY34962.1	5'-CTATTCGATGATGAAAGCC-3'
H5N2	NA	AF25743.1	5'-CGGAGCTTATGCTAGAC-3'
Seasonal A(H2N1)	NA	CY18725.1	5'-ACTATGAGAAAGCTCAAAAG-3'
Denkerm A(H2N1)	NA	JF825195.1	5'-ATGAAACAGGATATGTTCTG-3'
H2N2	NA	CY12896.1	5'-TTCAGAGGAAATGATGATG-3'
H2N8	NA	HM89208.1	5'-CTTGTTCAAAATGCCCTTA-3'
A(H2N2)	NA	CY11835.2	5'-AACTATGAAAGCAATGCTG-3'
H2N8	NA	HQ43881.1	5'-TTCAGGAAAGCTTAAAGGAA-3'
Seasonal A(H2N1)	NA	CY15326.1	5'-TCTCTTCACTCTACTA-A-3'
HPAI A(H2N1)	NA	EU24994.1	5'-ACTCTGAGCTGATGAGCTTT-3'
H7N7	NA	GU05311.1	5'-CCGCACTATGAGCAATGA-3'
H5N2	NA	AD178646.1	5'-CAAGAGGTTGAGAGAGGAA-3'
Seasonal A(H2N1)	NA	CY18723.1	5'-TCCCTTAAATACCGTTTAA-3'
Denkerm A(H2N1)	NA	JF825190.1	5'-TATGGAATGTTTCAACT-3'
H2N2	NA	CY12895.1	5'-AAACAAGCTGTAAGAGAGG-3'
H2N8	NA	HM89205.1	5'-ATCATGAGATTCCTCACTT-3'
A(H2N2)	NA	CY11833.2	5'-TTCAGAAAGCTTAAAGAA-3'
H2N8	NA	HQ43882.1	5'-CCCTTAAATACCGTTTAA-3'
Seasonal A(H2N1)	NA	CY15326.1	5'-TATCATGAGATTCCTCACT-3'
HPAI A(H2N1)	NA	EU24996.1	5'-TCCCTTAAATACCGTTTAA-3'
H7N7	NA	GU05311.1	5'-CCCTTAAATACCGTTTAA-3'
H5N2	NA	AD178646.1	5'-CTTAAATGCTTATGATG-3'
Seasonal A(H2N1)	PB1	CY12673.1	5'-AAGATGATGATGATGATG-3'
Denkerm A(H2N1)	PB1	HQ24702.2	5'-AGTGTGATGAAATCAAAATC-3'
H2N2	PB1	CY12890.1	5'-TGGATGAGATCAAAATCCT-3'
H2N8	PB1	HM89203.1	5'-GGATAAGAGAAATGAAAT-3'
A(H2N2)	PB1	CY11871.2	5'-TGTAACTTGGCCAAAAGAA-3'
H2N8	PB1	HQ43877.1	5'-GATCTATATCCAAAGACA-3'
Seasonal A(H2N1)	PB1	FF7381.1	5'-AGCATGAGCTGATGACTTA-3'
HPAI A(H2N1)	PB1	EU24997.1	5'-AGAAAATGDTACACAAAGAA-3'
H7N7	PB1	AY340083.1	5'-AATTAATGAGAAACCA-3'
H5N2	PB1	NC_048061.1	5'-AGTAAAGCACTGAGCTGAT-3'
Seasonal A(H2N1)	PA	CY12697.1	5'-AAACAGCACTTCAACCAAT-3'
Denkerm A(H2N1)	PA	HQ43878.1	5'-ATTCACCTGAGAGGAGTGG-3'
H2N2	PA	CY12692.1	5'-AGAAATCTCTTGGATGAAA-3'
H2N8	PA	HM89204.1	5'-GAAAGCCAGAGATATGCT-3'
A(H2N2)	PA	CY11858.2	5'-CAGAACCCTGATGATGAT-3'
H2N8	PA	HQ43879.1	5'-AGCCGAAAGAGCAATGAA-3'
Seasonal A(H2N1)	PA	CY15319.1	5'-TTCATGATGAGCAAGCTTT-3'
HPAI A(H2N1)	PA	EU24996.1	5'-CCGCAAGCTTCTCAAATG-3'
H7N7	PA	AY34011.1	5'-ACAACCAAGCCCTCTT-3'
H5N2	PA	NC_048062.1	5'-CTGAAATGAGCAATGAGAC-3'
Seasonal A(H2N1)	NP	CY12870.1	5'-GATGTAAGAAATGAGACAAA-3'
Denkerm A(H2N1)	NP	HQ24282.2	5'-TGTGTAATGAGAAATGCGG-3'
H2N2	NP	CY12891.1	5'-ACGAAATGTCGAAAGCA-3'
H2N8	NP	HM89209.1	5'-TATGGAAGATGACACATA-3'
A(H2N2)	NP	CY12697.1	5'-AGAACACTTGGTGAAGACT-3'
H2N8	NP	HQ43883.1	5'-TGCCTATGTAAGACTGCT-3'
Seasonal A(H2N1)	NP	CY15329.1	5'-ACCTCCACTCCTCCAAA-3'
HPAI A(H2N1)	NP	CY15811.1	5'-CCATGTAAGAAATGACGCA-3'
H7N7	NP	GU05312.1	5'-AAATGCAACAGGTTTAA-3'
H5N2	NP	FF73288.1	5'-TCCCTATGAAAGTCC-3'

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

**Electrochemistry rapid as method for identification influenza
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Results

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

3. Results:

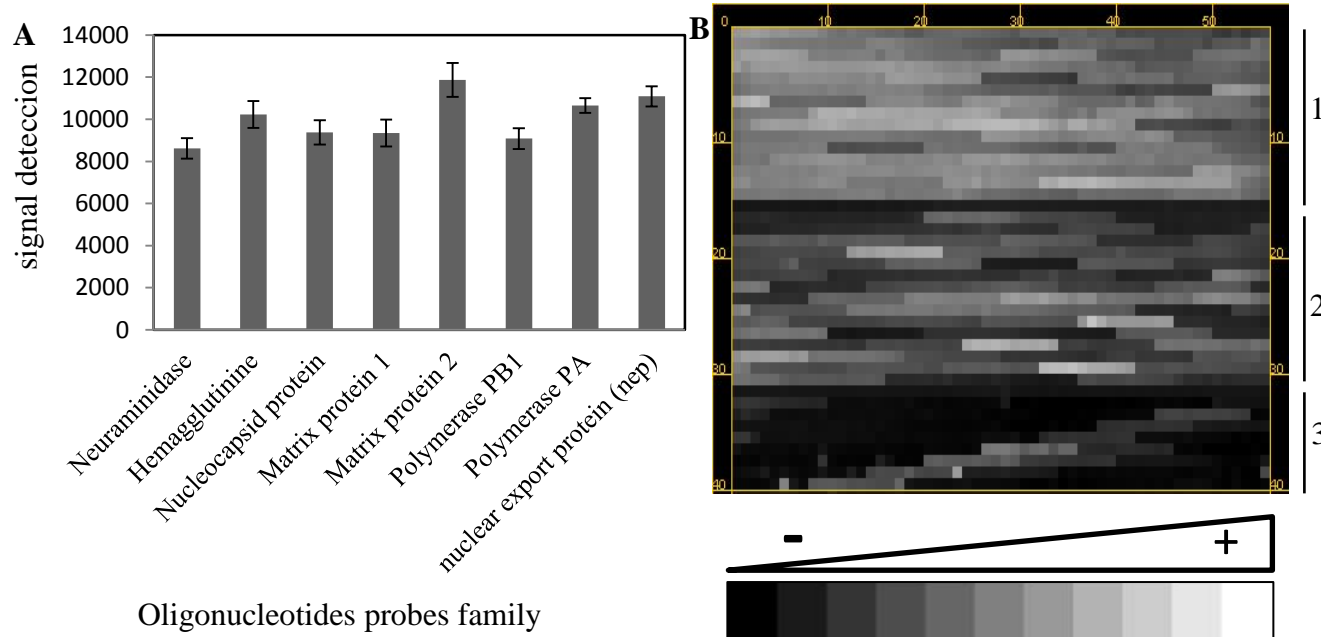


Figure 1. A) The average signal for probes oligonucleotides family and \pm error standard after hybridization. B) Image of microarray of influenza: 1) the position and intensity signal of 80 normal oligonucleotide probes, 2) 80 oligonucleotide probes with one mutation and 3) 32 oligonucleotide probes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

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3. Results:

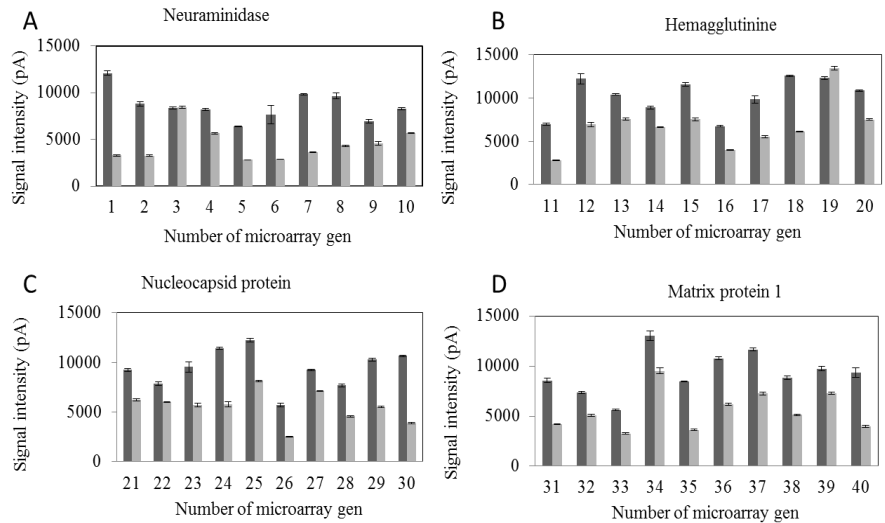


Figure 2. Signal after hybridization of oligonucleotide probes of influenza viruses without and/or one mutation in the sequences of probes. A) neuraminidase, B) hemagglutinine, C) nucleocapsid protein and D) matrix protein 1 genes.

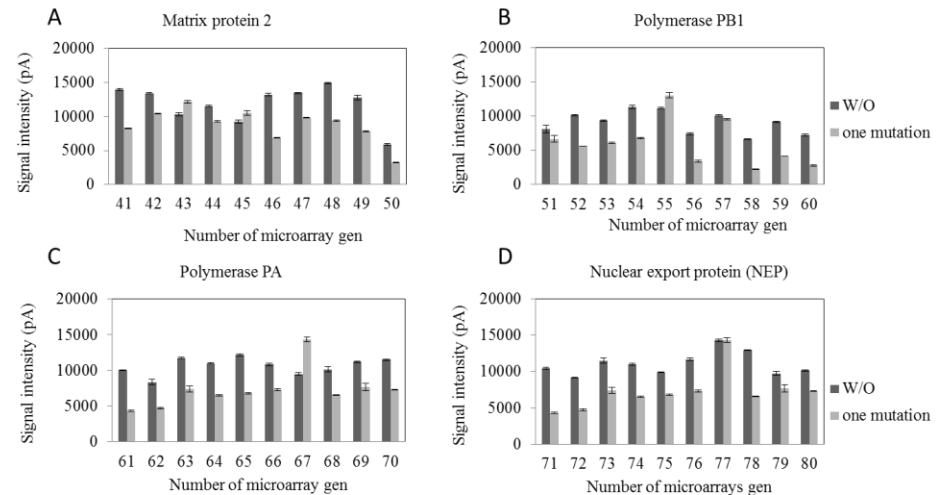
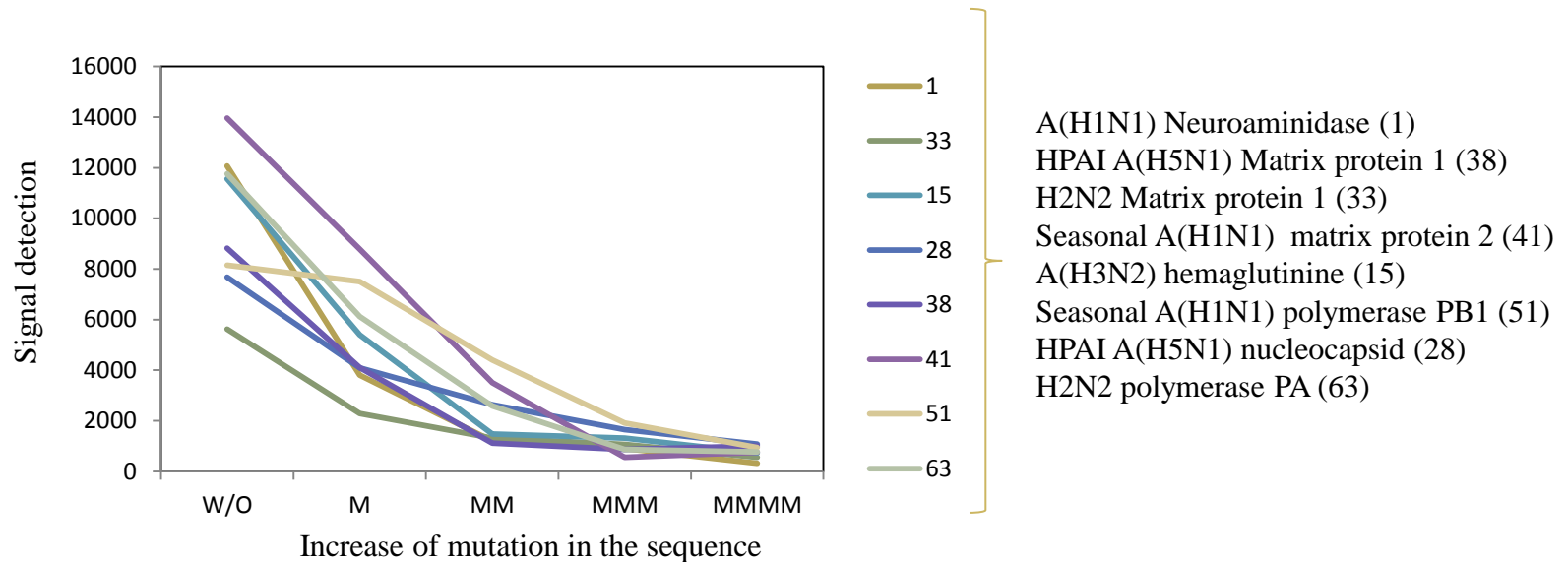


Figure 3. Signal after hybridization of oligonucleotide probes of influenza viruses without (W/O) and/or one mutation in the sequences of probes. A) matrix protein 2, B) polymerase PB1, C) Polymerase PA and D) nuclear export protein (NEP) genes

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

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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.

Thank you for your attention

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu