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

Název: **ELECTROCHEMISTRY METHOD FOR IDENTIFICATION INFLUENZA VIRUSES:**

CombiMatrix ElectraSense™

Školitel: Miguel Ángel Merlos Rodrigo

Datum: 12. 12. 2013

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet

CombiMatrix ElectroSense™: Microarray

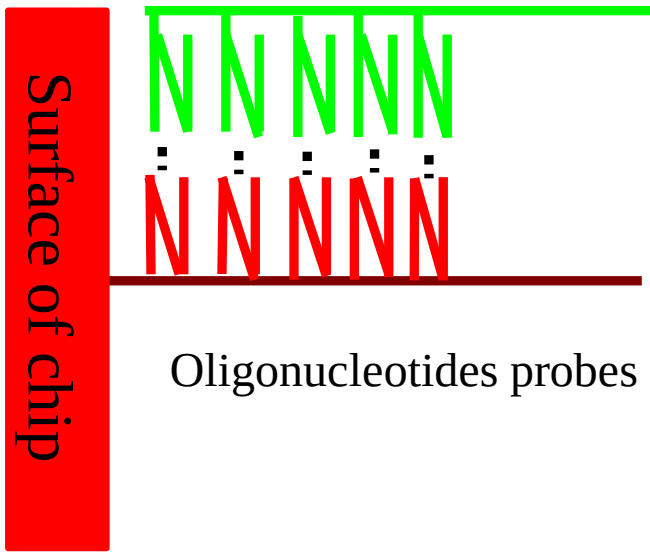
MicroArray is a new technology to show the expression of genes.

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

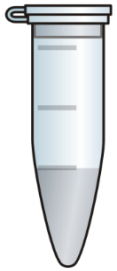
Array Reader

Chip

CombiMatrix ElectroSense™: Microarray and hybridization

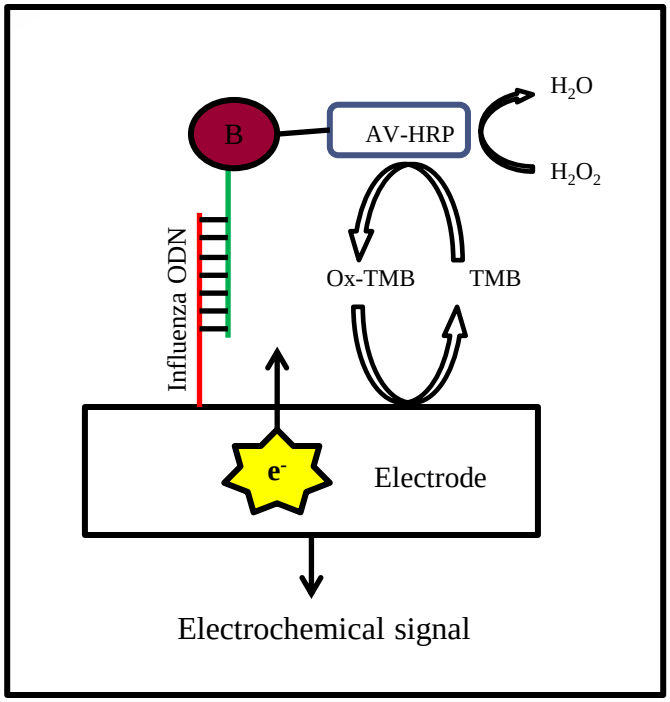


Oligonucleotides probes of influenza viruses inside of the chip



Samples of RNA Influenza viruses

CombiMatrix ElectroSense™: Electrochemical detection



B Biotin

AV-HRP
horseradish peroxidase

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

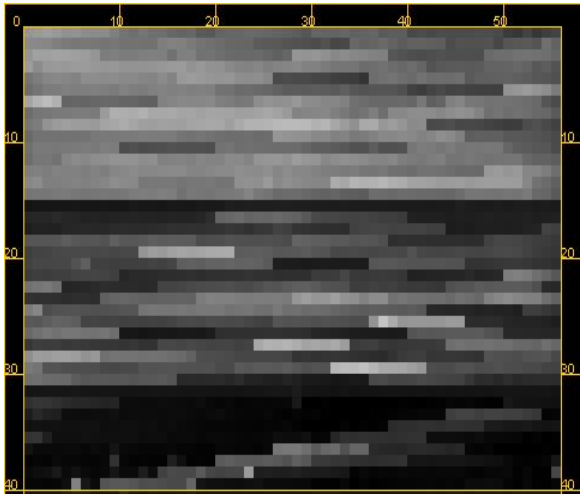
- The approach is based on the detection of redox active chemistries (such as horseradish peroxidase (HRP) and the associated substrate TMB) proximal to specific microarray electrodes.

- 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.

CombiMatrix ElectroSense™: Results



Viruses	Genes	GenBank accession number	Sequence	Number of position inside of Arrays
Seasonal A(H1N1)	neuraminidase	CY076797	5'-TCCTCATAATGAAAATTGGG-3'	1
Pandemic A(H1N1)	neuraminidase	GQ377078	5'-TCCTCATAGTGATAATTAGG-3'	2
H2N2	neuraminidase	CY125896.1	5'-TAGCAATTGCAGGGATCCTA-3'	3
H3N8	neuraminidase	HM589207.1	5'-TGGATCAAGAGGCCATGTTT-3'	4
A(H3N2)	neuraminidase	GQ293082	5'-ACACATAAGGTTCTCTGT-3'	5
H5N8	neuraminidase	JQ433881.1	5'-GTTGAAATCAGTTTCAATGG-3'	6
Seasonal A(H5N1)	neuraminidase	CY053327.1	5'-ACCGTTAAAGACAGAAGC C-3'	7
HPAI A(H5N1)	neuraminidase	EU263982.1	5'-AGGGAAAGTAGTTAAATCAG-3'	8
H7N7	neuraminidase	AY340079.1	5'-CCAGAAACATTCTGAGGACT-3'	9
H5N2	neuraminidase	NC_004909.1	5'-TACATGATAGGAGTCCCCAT-3'	10
Seasonal A(H1N1)	hemagglutinine	JN017181.1	5'-AGAGAAGAAATAGATGGGGTA-3'	11
Pandemic A(H1N1)	hemagglutinine	JN625498.1	5'-ACCCAAAGCTCAGCAAATC-3'	12
H2N2	hemagglutinine	CY125918.1	5'-TGTGTGGACATACAATGCTG-3'	13
H3N8	hemagglutinine	HM589205.1	5'-ATCAACACTAAATCAGAGGTC-3'	14
A(H3N2)	hemagglutinine	JX844665.1	5'-TTTTGTTGAACGCAGCAAAG-3'	15
H5N8	hemagglutinine	JQ433879.1	5'-GACCTTAAAAGCACTCAAGC-3'	16
Seasonal A(H5N1)	hemagglutinine	CY053325.1	5'-GGGAATACACCACCCTAAT-3'	17
HPAI A(H5N1)	hemagglutinine	EU263981.1	5'-CTCCAGAATATGCATACAAAA-3'	18
H7N7	hemagglutinine	AY338459.1	5'-GAAACGGTGGAACGAACAAA-3'	19
H5N2	hemagglutinine	NC_004908.1	5'-GTAGTGCAATGTCAGACTGA-3'	20
Seasonal A(H1N1)	nucleocapsid protein	CY087027.1	5'-ATAGACGGAAAATGGATGAG-3'	21
Pandemic A(H1N1)	nucleocapsid protein	JN625388.1	5'-GACGACTAATCCAGAATAGC-3'	22
H2N2	nucleocapsid protein	CY125897.1	5'-AGAGAGTAAATGGAAAGTGGA-3'	23
H3N8	nucleocapsid protein	HM589206.1	5'-AATGGAGAAGACGCAACC-3'	24
A(H3N2)	nucleocapsid protein	CY113840.2	5'-GATTATGAAGGGCGGTTGATT-3'	25
H5N8	nucleocapsid protein	JQ433880.1	5'-CTTCAGTCTTATCAGACCAAA-3'	26
Seasonal A(H5N1)	nucleocapsid protein	CY053328.1	5'-GCGAATAATGGAGAAGACG-3'	27
HPAI A(H5N1)	nucleocapsid protein	EU263985.1	5'-ACATATCAGAGAACGAGAGC-3'	28
H7N7	nucleocapsid protein	AY342425.1	5'-CTAATTCGGATGATAAAGCG-3'	29
H5N2	nucleocapsid protein	AF255743.1	5'-GGAAGGTTGATCCAGAAC-3'	30
Seasonal A(H1N1)	matrix protein 1	CY087025.1	5'-ACTATACAAGAAGCTCAAAAG-3'	31
Pandemic A(H1N1)	matrix protein 1	JN625390.1	5'-ATGAAAACAGAATGGTGCTG-3'	32
H2N2	matrix protein 1	CY125895.1	5'-TTAAGAGGGAGATAACATTC-3'	33
H3N8	matrix protein 1	HM589208.1	5'-CTTTGTCCAAAATGCCCTTAA-3'	34
A(H3N2)	matrix protein 1	CY113838.2	5'-AACATGTGAACAGATTGCTG-3'	35
H5N8	matrix protein 1	JQ433882.1	5'-TACAGGAAGCTTAAAGGGA-3'	36
Seasonal A(H5N1)	matrix protein 1	CY053326.1	5'-TCCTGTCACCTCTGACTA A-3'	37
HPAI A(H5N1)	matrix protein 1	EU263984.1	5'-ACTGCAGCGTAGACGTTTT-3'	38
H7N7	matrix protein 1	GU053111.1	5'-CCCACTAATCAGGCATGAA-3'	39
H5N2	matrix protein 1	AJ278646.1	5'-CAAGAAGCTGAAGAGGGAAA-3'	40
Seasonal A(H1N1)	matrix protein 2	CY087025.1	5'-TCGCTTTAAATACGGTTTGAA-3'	41
Pandemic A(H1N1)	matrix protein 2	JN625390.1	5'-TCATTGGGATCTTGCACCT-3'	42
H2N2	matrix protein 2	CY125895.1	5'-AACACGGTCTGAAAAGAGGG-3'	43
H3N8	matrix protein 2	HM589208.1	5'-ATCATTGGGATCTTGCACTT-3'	44
A(H3N2)	matrix protein 2	CY113838.2	5'-TTCAAACACGGTCTGAAAAGA-3'	45
H5N8	matrix protein 2	JQ433882.1	5'-GCCTTAAATACGGTTTGAA-3'	46
Seasonal A(H5N1)	matrix protein 2	CY053326.1	5'-TATCATTGGGATCTTGCACT-3'	47
HPAI A(H5N1)	matrix protein 2	EU263984.1	5'-TCGCCTTAAATACGGTTTGAA-3'	48
H7N7	matrix protein 2	GU053111.1	5'-CGCCTTAAATACGGTTTGAA-3'	49
H5N2	matrix protein 2	AJ278646.1	5'-CTTCAAATGCATTTATCGTC-3'	50
Seasonal A(H1N1)	polymerase PB1	CY125673.1	5'-AAGATGATGACCAACTCCCA-3'	51
Pandemic A(H1N1)	polymerase PB1	HQ240702.2	5'-AGTGGAATGAAAATCAAAATC-3'	52
H2N2	polymerase PB1	CY125900.1	5'-TGGAATGAGAATCAAAATCCT-3'	53
H3N8	polymerase PB1	HM589203.1	5'-GGATAAAGAGGAAATGGAAAT-3'	54
A(H3N2)	polymerase PB1	CY112971.2	5'-TGAATCTTGGGCAAAAGAAA-3'	55
H5N8	polymerase PB1	JQ433877.1	5'-GATGACTAATTCACAAGACA-3'	56
Seasonal A(H5N1)	polymerase PB1	JF758819.1	5'-AGCATTGACACTGAACACTA-3'	57
HPAI A(H5N1)	polymerase PB1	EU263987.1	5'-AGAAAATGGTACACAAAGAA-3'	58
H7N7	polymerase PB1	AY340083.1	5'-AATTACTGAGACAACACCA-3'	59
H5N2	polymerase PB1	NC_004911.1	5'-AGTAAGAGCATGAAGCTACG-3'	60
Seasonal A(H1N1)	polymerase PA	CY125671.1	5'-AAACCAGACTATTCACCATAA-3'	61
Pandemic A(H1N1)	polymerase PA	JQ433878.1	5'-ATTCACTGGAGAGGAGATGG-3'	62
H2N2	polymerase PA	CY125923.1	5'-AGAACATTCTTTGGATGGAA-3'	63
H3N8	polymerase PA	HM589204.1	5'-GAAAGCCAACAAGATAAAATC-3'	64
A(H3N2)	polymerase PA	CY113858.2	5'-CAGAGCCACTGAGTACATAAT-3'	65
H5N8	polymerase PA	JQ433878.1	5'-AGGCGAAGAGACAATTGAAG-3'	66
Seasonal A(H5N1)	polymerase PA	CY053330.1	5'-TGCATTGAGGGCAAGCTTT-3'	67
HPAI A(H5N1)	polymerase PA	EU263986.1	5'-GGGCAAGCTTTCTCAAATGT-3'	68
H7N7	polymerase PA	AY342418.1	5'-ACAACACCACGCCCTCT-3'	69
H5N2	polymerase PA	NC_004912.1	5'-CTGAAATTAAGCATTGAGGAC-3'	70
Seasonal A(H1N1)	nuclear export protein (nep)	CY125670.1	5'-GATTGAAGAAGTGAGACACAA-3'	71
Pandemic A(H1N1)	nuclear export protein (nep)	HQ240288.2	5'-TGGTTAATTGAAGAAATGCGG-3'	72
H2N2	nuclear export protein (nep)	CY125898.1	5'-ACGGAAAATGGCGAGAACAA-3'	73
H3N8	nuclear export protein (nep)	HM589209.1	5'-TGATTGAGGAAGTACGACATA-3'	74
A(H3N2)	nuclear export protein (nep)	CY103967.1	5'- AGAACAGTTAGGTCAAAAGT-3'	75
H5N8	nuclear export protein (nep)	JQ433883.1	5'-TGGCTGATTGAAGAAGTGC-3'	76
Seasonal A(H5N1)	nuclear export protein (nep)	CY053329.1	5'-ACCTCCACTCCCTCCAAAA-3'	77
HPAI A(H5N1)	nuclear export protein (nep)	CY099818.1	5'-GCTGATTGAAGAAGTACGACA-3'	78
H7N7	nuclear export protein (nep)	GU053125.1	5'-AAGTGCGACATAGGTTGAAG-3'	79
H5N2	nuclear export protein (nep)	FJ793288.1	5'-TGGCTGATTGAAGAAGTGC--3'	80

CombiMatrix ElectroSense™: Review of Results

Kristian M. Roth et al, demonstrated very good detection of avian influenza subtype H5N1 by application of electrochemical detection of Combimatrix microarrays

Electrochemical Detection of Short DNA Oligomer Hybridization Using the CombiMatrix ElectraSense Microarray Reader

Kristian M. Roth, Kia Peyvan, Kevin R. Schwarzkopf, Andrei Ghindilis*

Combimatrix Corp., 6500 Harbour Heights Pkwy Mukilteo, WA 98275
*e-mail: kroth@combimatrix.com

Michael J. Lodes et al, identified of upper respiratory tract pathogens by electrochemical detection on an oligonucleotide microarray, including A and B influenza viruses

PLOS ONE

Identification of Upper Respiratory Tract Pathogens Using Electrochemical Detection on an Oligonucleotide Microarray

Michael J. Lodes[1]*, Dominic Suciu[1], Jodi L. Wilmoth[1], Marty Ross[1], Sandra Munro[1], Kim Dix[1], Karen Bernards[1], Axel G. Stöver[1], Miguel Quintana[2], Naomi Iihoshi[2], Wanda J. Lyon[3], David L. Danley[1], Andrew McShea[1]

1 CombiMatrix Corporation, Mukilteo, Washington, United States of America, 2 United States Army Center for Health Promotion and Preventive Medicine-West, Fort Lewis, Washington, United States of America, 3 Air Force Research Laboratory/Human Effectiveness Directorate, Applied Biotechnology Branch, Wright-Patterson Air Force Base, Ohio, United States of America

CombiMatrix ElectroSense™: Review of Results

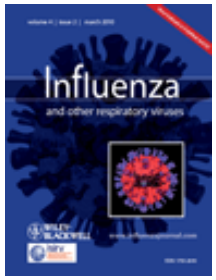
Shelly Bolotin et al, showed that the sensitivity of the CombiMatrix influenza detection system was 95.2% and the specificity was 100% for influenza A subtype during the 2007–2008 influenza season in Toronto, Canada

VIROLOGY JOURNAL

Verification of the Combimatrix influenza detection assay for the detection of influenza A subtype during the 2007–2008 influenza season in Toronto, Canada

Shelly Bolotin*[1], Ernesto Lombos[1], Rani Yeung[1], AliReza Eshaghi[1], Joanne Blair[1] and Steven J Drews[1,2,3]

Straight, T.M. et al, correctly identified 23 of 24 samples of laboratory-confirmed pandemic (H1N1) 2009 Influenza by the ElectraSense Influenza A assay.



A novel electrochemical device to differentiate pandemic (H1N1) 2009 from seasonal influenza

T. M. Straight1, G. Merrill1,L. Perez1, J. Livezey1, B. Robinson1,M. Lodes2,D. Suciu2,B. Anderson2

CombiMatrix ElectroSense™: Conclusions

The CombiMatrix influenza detection system is an effective methodology for influenza A and B subtype analysis

Rapid identification pathogens will significantly decrease the time and cost for the identification of potential lethal virus and bacterial strains and lead to better treatment and management of infections

Microarray and biosensor technologies show great promise for virus and bacteria detection and genotyping and are needed for rapid effective treatment, environmental monitoring and the detection of bioterrorism agents.

CombiMatrix ElectroSense™: **Acknowledgements**

I want to thank all colleagues of the laboratory

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CZ.1.07/2.4.00/31.0023 is highly acknowledged

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Thank you for attention

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet