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14. ABSTRACT
Major histocompatibility complex (MHC), human leukocyte antigen (HLA) in humans, plays significant role in mate selection and kin recognition mainly through apparition of organism specific odor recognized by other individuals. However a mechanism of the relationship between unique MHC genetic combination of an organism and generation of the specific organism odor is not understood. We are showing here that human B cells produce volatile organic (odor) compounds measurable by GC/MS technique. More importantly, our results evidence that a presence of specific HLA allele in the cells is related to apparition of specific cell odor fingerprint. This work demonstrates for the first time that HLA influences production of specific odor

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Report Title

Characterization of MHC-related volatile organic compounds in heterologous expression systems: role of infection in odor compound generation

ABSTRACT

Major histocompatibility complex (MHC), human leukocyte antigen (HLA) in humans, plays significant role in mate selection and kin recognition mainly through apparition of organism specific odor recognized by other individuals. However a mechanism of the relationship between unique MHC genetic combination of an organism and generation of the specific organism odor is not understood. We are showing here that human B cells produce volatile organic (odor) compounds measurable by GC/MS technique. More importantly, our results evidence that a presence of specific HLA allele in the cells is related to apparition of specific cell odor fingerprint. This work demonstrates for the first time that HLA influences production of specific odor compounds already on cellular level (in a single cell) and that the cell odor fingerprint depends on expression of specific HLA molecules.

Subsequently, we have investigated how infection of cell cultures influences odor compound generation. We have evidence that when cells are infected with three different influenza strains that they produce different odor profiles. These findings are of significance for future applications in non-invasive and minimally-invasive diagnostics. Monitoring for these infection associated compounds in human breath may provide an avenue for rapid mass triage of infected patients from emerging infectious diseases.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Number of Papers published in peer-reviewed journals: 0.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

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Individual body odors. Army Research Office Workshop: Trace Gas Detection by Artificial, Biological and Computational Olfaction. Monell Chemical Senses Center, March 29-31, 2006

Number of Presentations: 1.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Davis CE, Ayer S, Bhushan A, Bissell J, Boehme S, Han H, Loyola B, Molina MA, Nielsen W, Pringle D, Pulford S, Sankaran S, Smith R, Zhao W. Microsystems Sensors and Actuators for Healthcare. National Academies Keck Futures Initiative: "The Future of Human Healthspan: Demography, Evolution, Medicine and Bioengineering." Irvine, CA, November 13-16, 2007.

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 1

Peer-Reviewed Conference Proceeding publications (other than abstracts):

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(d) Manuscripts

Cardona CJ, Xing Z, Sandrock CE, Davis CE. (2007) Avian influenza in birds and animals. Journal of Clinical Microbiology [invited review]

Zhao W, Morgan JT, Davis CE. (2007) Chromatogram Classification Based on Complex Coefficients of an Autoregressive Model.

Gojova A, Morgan JT, Sankaran S, Zhao W, Davis CE. Human leukocyte antigen (HLA) genes associated with generation of volatile organic compounds at a cellular level.

Morgan JT, Sankaran S, Gojova A, Zhao W, Davis CE*. High throughput metabolomic screening of mammalian cells using gas chromatography mass spectrometry GC/MS.

Number of Manuscripts: 4.00

Number of Inventions:

Graduate Students

NAME	PERCENT_SUPPORTED
Joshua Morgan	0.50
Stephanie Pulford	0.50
FTE Equivalent:	**1.00**
Total Number:	**2**

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NAME	PERCENT_SUPPORTED
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Weixiang Zhao	0.60
FTE Equivalent:	**1.10**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Cristina Davis	0.08	No
FTE Equivalent:	**0.08**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
Mary Molina	0.00
FTE Equivalent:	**0.00**
Total Number:	**1**

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	1.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	1.00
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The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	1.00

Names of Personnel receiving masters degrees

NAME
Joshua Morgan
Total Number: 1

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED	
Shankar Sankaran	1.00	No
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Characterization of MHC-related volatile organic compounds in heterologous expression systems: role of infection in odor compound generation.

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Final Report

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Report Period Final

November 2007

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1.0 Executive Summary:

1. We optimized measurement conditions to study volatile organic compounds produced in cell culture based systems using gas chromatography mass spectrometry.
2. Using specialized B-cell lines, we were able to study the influence of specific human leukocyte antigen (HLA) alleles on odor compound generation.
3. Specific HLA alleles correlated with specific odor compound up and down regulation.
4. Infection of cell cultures by three influenza strains produced marked odor compound differences, which may yield avenues for future non-invasive viral diagnostics in the future.

2.0 Technical progress of HLA associated odor experiments.

Major histocompatibility complex (MHC), in humans referred to as human leukocyte antigen (HLA) complex, is extraordinarily genetically diverse which provides each organism with its immunological individuality. The existence of MHC diversity is traditionally thought to be crucial for protection of species against lethal pathogens. More recently, a MHC function in mate selection and kin recognition was largely studied. As it became established the immunological individuality of an organism is also apparent externally as a specific odor signature of the organism. {Yamazaki, 1976 #8}{Yamazaki, 1983 #1}{Yamaguchi, 1981 #2}{Singh, 1987 #3} The phenomenon of individual organism odor related to MHC system of the organism was the most extensively studied in mice {Yamazaki, 1976 #8}{Yamazaki, 1979 #9}{Willse, 2006 #16} {Beauchamp, 2003 #17} however it is also present in other species and in some forms in humans {Wedekind, 1995 #4} {Eggert, 1998 #10} {Wobst, 1998 #11} {Jacob, 2002 #18}. These studies showed evidence of a relationship between a specific odor compounds production related to a specific combination of MHC antigens. However, a precise mechanism how a given MHC combination leads to this specific odor appearance is not resolved. The most widespread hypothesis – a “carrier hypothesis” – proposes that metabolites of MHC molecules (soluble MHC) cleaved from membrane-bound MHC are the carriers of volatiles. {Singh, 1987 #3}{Singh, 1998 #13} On the contrary to this and other hypotheses, we have hypothesized that the MHC influences production of volatile

organic compounds (VOCs) **directly on a cellular level.** In other words, specific MHC combination influences metabolic pathways and therefore production of VOCs of each single cell of a multicellular mammalian organism. This is an entirely novel view attempting to explain the MHC-odor relationship. In the past, the VOC presence and composition was measured in mammalian urine or blood {Singer, 1997 #14} {Cavaggioni, 2006 #19} {Eggert, 1998 #10} {Schaefer, 2002 #20} {Willse, 2006 #16} however never in single cells. Additionally in this study, we have attempted to track specific VOC production related to a single HLA class I allele.

In order to test our hypothesis we have studied production of VOCs in a human cell line using gas chromatography coupled with mass spectrometry (GC/MS). To establish VOC production dependent on a presence or absence of a single HLA class I allele we tested a human B-lymphoblastoic C1R class I null cell line and several single HLA class I transfectants of C1R cell line (generous gift of Dr. Peter Cresswell, Yale University). HLA class I molecules are expressed in virtually every nucleated cell of human body therefore are suitable target to test our hypothesis. Our results show that it is possible to measure specific odor fingerprint of mammalian cells. More importantly, we are presenting here for the first time that the specific HLA allele presence influences production of VOCs and therefore whole-cell specific odor fingerprint.

We have used the C1R cell line and four different single HLA class I transfectants of C1R – C1R A2, C1R A69, C1R B7, and C1R B27. The C1R cell line is a human B-lymphoblastoic HLA class I A and B locus negative cell line. {Storkus, 1989 #6} {Storkus, 1987 #5} It does express small amounts of HLA Cw4. The C1R transfectants each express single HLA class I allele that was added back to C1R null background. {Storkus, 1991 #7} All the cell lines used in this study were generously provided to us by Dr. Peter Cresswell, Yale University. They are described in the literature and their nature is fully confirmed. We have additionally tested presence of all HLA alleles we have studied in the cell lines using Western Blot procedure. We have confirmed that each cell line expresses only the expected HLA allele and does not express the alleles of other tested cell lines (Figure 1). Therefore, while testing odor composition of these cell lines under completely identical cellular and experimental conditions the expected difference in VOC production would be related solely to presence of a specific HLA allele.

It is well known that the metabolism of mammalian cells is varied and complex. Numerous methods have been utilized in characterizing metabolic activity, but generally have limited throughput. Gas chromatography coupled with mass spectrometry is a high throughput method which both separates complex samples and provides repeatable and unique compound identification. Using GC/MS we have tested a composition of a headspace gas phase above the human B-lymphoblastoic cell suspension. In the past our group successfully used similar technique to establish composition of headspace above different kinds of bacteria {Shnayderman, 2005 #15} {Krebs, 2006 #21}, however there are no studies using this technique for mammalian cell culture. In separate experiments, each C1R or single HLA transfectant of C1R cell line was seeded into tightly sealed glass vials at a concentration of 800,000 cells/ml in serum-free cell culture medium. After a 24 hour-incubation period at 37°C the VOCs present in the headspace were adsorbed to a gray SPME fiber. Gray SPME fibers are optimized for non-polar molecules in the 40-275 molecular weight range, although they do absorb outside of this range as well. As expected, our analysis favored midsized non-polar molecules, such as aromatic ring

structures, although other compounds were detected as well. GC/MS analysis of adsorbed compounds produced highly repetitive chromatograms that were specific for each cell line (Figure 2A). Figure 2A demonstrates the high information content that SPME headspace sampling provides. Each peak represents a unique compound that was present in the headspace above the cell culture and has associated m/z spectra which can be used to identify the compound. By studying the presence, absence and amplitudes of the volatiles and semi-volatiles, information about the condition and metabolic activity of the cells can be inferred. The largest peaks in the spectra tend to come from instrument background (column and septa bleed) or from culture media (for example preservatives used in the serum). These peaks can have total ion counts (TICs) on the order of $1x10^6$ counts, which is two orders of magnitude higher then the lowest intensity peaks used for cell line differentiation, which could be on the order of $1x10^4$.

Closer look at the chromatograms show clear differences in presence or amplitude of certain peaks. In summary, three different situations happened when comparing peaks of two cell lines. Figure 2B describes these three situations. Situation of high significant peak versus low significant peak, significant peak versus weak peak, and peak vs. non-peak (Figure 2B)

We performed detailed analysis of GC/MS data to describe differences among tested cell lines. Table 0 shows thirty peaks that can be used to separate cell lines. The retention times and peak amplitudes of twelve samples of each cell line were used to calculate the mean retention time and mean amplitude and their standard deviations. Twelve peak amplitudes of each of any two cell line were also used to calculate the p-value to determine if these two cell lines are separable. It can be seen from this table that (1) the best peaks are peaks 15 and 20 that are able to separate 10 pairs of cell lines (Six cell lines totally yield 15 pairs of cell lines) and (2) the numbers of peaks that are able to separate 15 pair of cell lines (C1RA2-C1RA68, C1RA2-C1RA69, C1RA2-C1RB7, C1RA2-C1RB27, C1RA2-C1R, C1RA68-C1RA69, C1RA68-C1RB7, C1RA68-C1RB27, C1RA68-C1R, C1RA69-C1RB7, C1RA69-C1RB27, C1RA69-C1R, C1RB7-C1RB27, C1RB7-C1R, C1RB27-C1R) are 27, 14, 10, 5, 18, 14, 13, 17, 9, 2, 11, 9, 6, 4, and 5, respectively. Table 1 provides a convincible evidence to show the good separation of six cell lines based on their GC/MS data. These results clearly show that presence of specific HLA allele leads to apparition of specific cell odor fingerprint. Given that cell numbers were tightly controlled during all experiments and experimental protocol was completely identical among different runs and it included control of cell viability, we believe that the differences in peak amplitudes between two cell lines are attributed to specific HLA allele presence and therefore its effect on cell metabolism leading to production of specific VOC fingerprint. More important argument supporting our data and conclusions is that we recorded significant amount of situations when a compound is completely absent in one cell line while present in another cell line expressing different HLA allele. Another important conclusion is that the identified VOCs produced by cells were released from cell inside to the headspace gas phase. This fact would favor a conclusion of VOC release produced by each cells also on a level of whole organism. These VOCs would then form whole organism odor or at least be responsible for its part.

Additionally, we attempted to identify these HLA-specific odor peaks showing differences in presence or in amplitude. These peaks represent specific odor signature of each cell line dependent on expression of certain HLA allele. The results of this study

suggest that it is possible to track HLA related volatile organic compounds to single human cells. Therefore the cell specific HLA composition influences cell metabolic pathways leading to production of specific odor compounds. This novel mechanism described here may be at least partially responsible for formation of whole organism odor proved to be dependent on HLA.

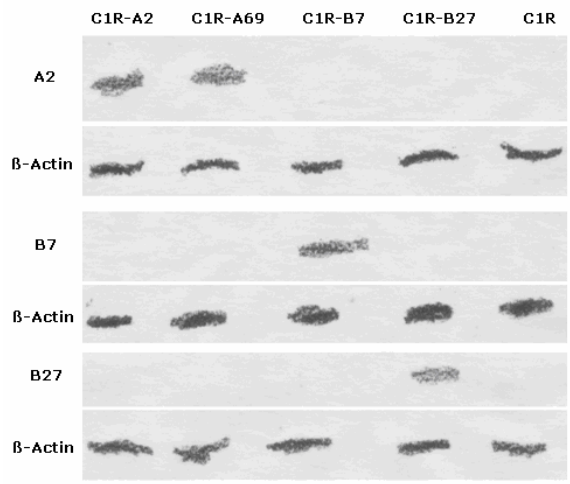
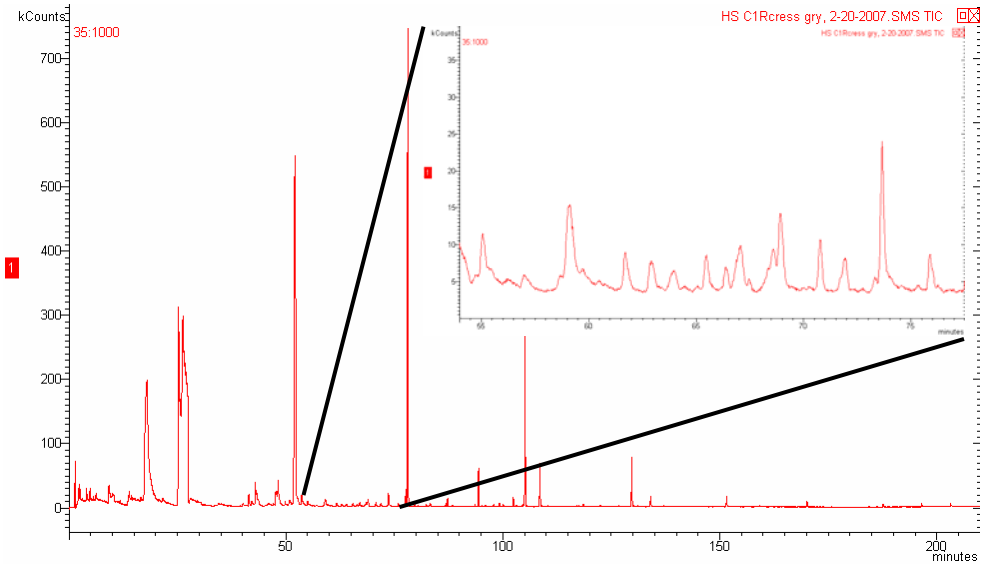


Figure 1: Analysis of HLA allele presence in all C1R cell lines used of protein isolated from each cell line used in this study was analyzed on a 10% acrylamide/bisacrylamide gel. Presence of HLA A2 and HLA A69 alleles was detected using anti-HLA A2 monoclonal antibody, presence of HLA B7 and HLA B27 alleles by anti-HLA B7 and HLA B27 monoclonal antibodies, respectively. Each cell line was tested for all antigens. ß-actin was used as loading control. Representative photographs are shown.

2A



2B

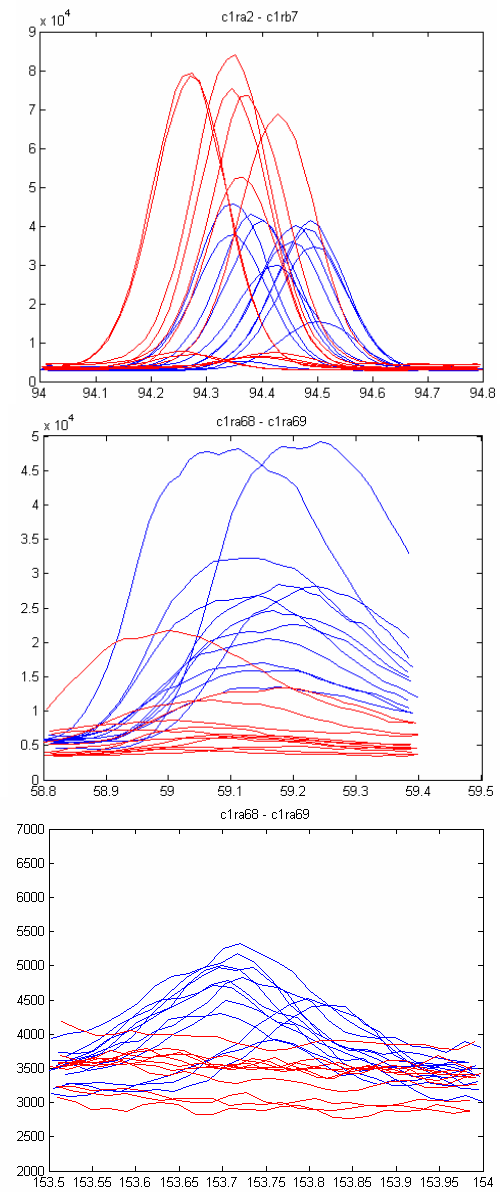


Figure 2: Volatile organic compound production analysis of C1R cell lines using gas chromatography/mass spectrometry. The headspace composition of 800,000 cells/ml was analyzed by GC/MS. The representative GC/MS chromatogram showing headspace composition of C1R cell line is presented in Fig. 2A. Entire chromatogram is shown with zoom-in window detailing cell line specific peaks that were later analyzed. Fig. 2B. shows three typical separable peak distributions encountered during data analysis. Upper panel represents situation of high significant peak versus low significant peak, medium panel of significant peak versus weak peak, and bottom panel of peak vs. non-peak.

Table 0. Differences in production of volatile organic compounds among tested cell lines. Cell line peak distribution comparison were based on retention time and peak amplitude [R.T.: retention time, A.: amplitude, S.P.: separable peaks ($p<0.05$). Two cell lines that have a same letter in their S.P. column are separable. For example, for peak 1, both C1RA2 and C1RA68 have letter 'a' in their S.P. columns, so they are separable in terms of peak 1.), '--': no peak, (*): standard deviation].

Peak	C1RA2			C1RA68			C1RA69			C1RB7			C1RB27			C1R		
No.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.
1	53.78 (0.08)	12935 (5543)	ade	53.66 (0.05)	17459 (3203)	af	53.67 (0.15)	10942 (8300)	fkl	53.59 (0.10)	12281 (8881)	mn	53.72 (0.09)	20498 (10184)	dkm	53.71 (0.10)	20170 (9731)	eln
2	59.14 (0.04)	30170 (27085)	b	59.17 (0.04)	27267 (11444)	fgh	59.06 (0.08)	8632 (4897)	bfkl	59.05 (0.08)	15568 (14256)	g	59.16 (0.09)	14440 (7149)	hk	59.14 (0.07)	21499 (19024)	l
3	61.79 (0.06)	6057 (1629)	ade	61.74 (0.04)	8230 (955)	afg	61.71 (0.13)	5957 (2314)	fkl	61.70 (0.11)	6273 (2575)	gn	61.78 (0.07)	8382 (3016)	dk	61.79 (0.05)	8857 (3077)	eln
4	62.95 (0.07)	6068 (1226)	ae	62.96 (0.06)	8443 (1128)	afg	62.85 (0.10)	5635 (2484)	fl	62.88 (0.08)	6337 (2794)	g	62.93 (0.13)	8838 (4982)		62.95 (0.10)	10048 (6091)	el
5	63.98 (0.05)	5047 (823)	a	64.04 (0.05)	5995 (473)	a	63.87 (0.08)	5635 (900)		63.90 (0.07)	5615 (948)		64.03 (0.08)	5686 (1055)		64.02 (0.07)	5785 (931)	
6	65.50 (0.05)	6581 (863)	ae	65.53 (0.04)	7799 (1221)	af	65.41 (0.06)	6836 (830)	f	65.43 (0.07)	6932 (1557)		65.52 (0.06)	7262 (2073)		65.52 (0.07)	8098 (2380)	e
7	66.43 (0.06)	6151 (1305)	a	66.43 (0.04)	8800 (2090)	afh	66.27 (0.08)	5997 (2565)	f	66.32 (0.08)	6877 (2854)		66.44 (0.07)	6648 (2829)	h	66.41 (0.09)	8323 (3671)	
8	67.12 (0.09)	7566 (1204)	abce	67.11 (0.08)	11986 (2162)	ah	66.96 (0.07)	9965 (2746)	b	67.00 (0.09)	10254 (2930)	c	67.12 (0.07)	8480 (3130)	h	67.13 (0.07)	10862 (4342)	e
9	73.67 (0.05)	13627 (3011)	b	73.67 (0.06)	17341 (9549)		73.59 (0.05)	19248 (6615)	b	73.60 (0.06)	20671 (14468)		73.69 (0.06)	15499 (9144)		73.69 (0.06)	21171 (12442)	
10	75.89 (0.05)	6295 (745)	abce	75.92 (0.03)	8464 (1506)	a	75.81 (0.05)	7784 (1058)	b	75.82 (0.06)	7623 (1917)	c	75.92 (0.06)	7133 (2052)		75.91 (0.07)	8228 (2475)	e
11	79.72 (0.05)	4232 (391)	abce	79.75 (0.04)	5622 (450)	ah	79.64 (0.05)	5909 (896)	bk	79.64 (0.06)	5299 (912)	c	79.76 (0.06)	4760 (837)	hk	79.74 (0.06)	5275 (1158)	e
12	82.40 (0.07)	4402 (951)	ab	82.43 (0.04)	6647 (1292)	afghi	82.27 (0.06)	5364 (1144)	bf	82.30 (0.07)	5311 (1327)	g	82.44 (0.08)	4935 (787)	h	82.43 (0.10)	5297 (1078)	i
13	86.89 (0.06)	6368 (922)	ae	86.92 (0.04)	7836 (1429)	a	86.78 (0.10)	6774 (1419)		86.81 (0.07)	7159 (1854)		86.93 (0.08)	6979 (2130)		86.92 (0.08)	7925 (2424)	e
14	91.59 (0.09)	3323 (362)	abce	91.64 (0.03)	4383 (490)	ahi	91.51 (0.08)	4164 (598)	bkl	91.53 (0.08)	4296 (1069)	cm	91.62 (0.06)	3580 (296)	hkm	91.66 (0.08)	3722 (272)	eil
15	92.10 (0.07)	3479 (524)	abde	92.12 (0.05)	4672 (857)	agh	92.01 (0.05)	4553 (821)	bjk	91.90 (0.03)	3728 (577)	gjm	--	--	dhkmo	92.13 (0.07)	4100 (796)	eo
16	94.43 (0.06)	34152 (12063)	ae	94.46 (0.04)	68965 (19210)	afh	94.33 (0.06)	35291 (35196)	fl	94.35 (0.06)	45589 (34931)		94.46 (0.07)	44974 (20992)	ho	94.46 (0.07)	67162 (29740)	elo
17	97.92 (0.05)	5105 (669)	abce	97.96 (0.04)	7410 (1535)	ah	97.85 (0.05)	6943 (1054)	b	97.86 (0.06)	6879 (2071)	c	97.96 (0.06)	5942 (1680)	h	97.96 (0.07)	6872 (2099)	e
18	99.20 (0.05)	6352 (966)	abce	99.24 (0.04)	9229 (2024)	ahi	99.13 (0.05)	8679 (1642)	bk	99.14 (0.06)	8397 (2760)	c	99.25 (0.06)	6900 (1587)	hk	99.24 (0.06)	7507 (1359)	ei
19	100.15 (0.07)	4626 (784)	abce	100.18 (0.04)	6554 (1193)	ah	100.07 (0.05)	6353 (1156)	b	100.08 (0.06)	6063 (1850)	c	100.18 (0.06)	5335 (1619)	h	100.17 (0.07)	6422 (2681)	e

20	102.45 (0.08)	10217 (4695)	ade	102.46 (0.04)	16049 (6194)	afg	102.34 (0.05)	8818 (2425)	fkl	102.35 (0.06)	9468 (4124)	gmn	102.48 (0.09)	13905 (3577)	dkmo	102.50 (0.10)	19636 (7862)	elno
21	117.50 (0.06)	3873 (371)	abc	117.52 (0.05)	4812 (606)	ahi	117.40 (0.05)	4771 (605)	bkl	117.42 (0.06)	4687 (967)	cm	117.54 (0.06)	3959 (518)	hkm	117.53 (0.07)	4169 (372)	il
22	118.63 (0.06)	5112 (778)	abce	118.65 (0.07)	7024 (1803)	a	118.55 (0.05)	6753 (1187)	b	118.56 (0.06)	6672 (1960)	c	118.68 (0.07)	5881 (2135)		118.66 (0.07)	6559 (1965)	e
23	122.46 (0.06)	4455 (768)	ae	122.50 (0.04)	5313 (1011)	ag	122.36 (0.06)	4648 (564)	l	122.38 (0.07)	4536 (766)	gn	122.50 (0.07)	4790 (429)	o	122.49 (0.07)	5351 (666)	elno
24	134.08 (0.06)	13626 (4647)		134.12 (0.03)	17820 (6484)	g	134.00 (0.07)	26179 (24573)	j	134.00 (0.05)	10654 (5164)	gj	134.12 (0.07)	14884 (10046)		134.11 (0.06)	41466 (84456)	
25	--	--	a	145.61 (0.05)	4602 (928)	afghi	--	--	f	--	--	g	--	--	h	--	--	i
26	--	--	a	153.73 (0.04)	4706 (396)	afghi	--	--	f	--	--	g	--	--	h	--	--	i
27	--	--	a	159.67 (0.05)	5182 (549)	afghi	--	--	f	--	--	g	--	--	h	--	--	i
28	--	--	a	160.09 (0.05)	4555 (382)	afghi	--	--	f	--	--	g	--	--	h	--	--	i
29	163.05 (0.06)	3702 (357)	ad	163.08 (0.04)	5542 (944)	afghi	162.99 (0.16)	4395 (1113)	fk	163.00 (0.11)	3802 (704)	gm	163.08 (0.12)	3294 (284)	dhkmo	163.05 (0.10)	3881 (772)	io
30	196.57 (0.03)	4775 (538)	abce	196.61 (0.05)	6510 (1763)	a	196.54 (0.03)	7626 (1877)	bk	196.54 (0.03)	6347 (1810)	c	196.58 (0.03)	5259 (1417)	k	196.58 (0.03)	6208 (1499)	e

3.0 Technical progress of infection experiments.

Headspace sampling virus infected cells experiments:

We have finished 12 repetitions of each set of virus infections for both 24 and 48 hour time points. Our previous results demonstrated a clear difference in GC profile for infected versus non-infected cells as well differences between different strains. The revamped results below include extra data collected as well as contain a complete statistical analysis of the previous data, with stricter parameters. The statistical results for these two groups of samples are listed in Table (1 and 2). T-test was used to determine if two cell lines have significantly different mean amplitude values at each peak. The 24-hour infected samples have 30 peaks ($p<0.05$) that can separate one cell line from the others while the 48-hour infected samples have 34 such peaks. The chemical identification of the common peaks for both 24-hr and 48-hr samples and the corresponding match factor of each cell line are listed in Table (3).

Table 1: Peak distribution comparison in terms of retention time and peak amplitude for viral infected cell lines **(24 Hrs)**

['-C1, -C2, …': the first, second, … common peaks shared wit 48-hr infected cell lines. R.T.: retention time (min.). A.: amplitude (total ion counts, TIC), S.P.: separable peaks ($p<0.05$. Any two cell lines that have a same letter in their S.P. column are separable. '--': either no peak or not detected by the peak criteria. (*): standard deviation].

Peak No.	Cntrl			MOI 1 H1N1			MOI 10 H1N1			MOI 10 H6N2			MOI 10 H9N2		
	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.
1	1.43 (0.04)	146783 (134582)		1.44 (0.03)	187363 (103803)	g,	1.46 (0.00)	182485 (101271)		1.46 (0.00)	226571 (154656)	j,	1.46 (0.01)	112734 (62588)	g, j,
2-C1	1.92 (0.12)	10047 (3800)	a, b, c, d,	2.05 (0.04)	18975 (5286)	a, e, f, g,	2.07 (0.01)	34638 (7187)	b, e, h, i,	2.06 (0.01)	53071 (6207)	c, f, h, j,	2.07 (0.00)	27768 (4821)	d, g, i, j,
3	2.50 (0.05)	9148 (1859)	d,	2.51 (0.04)	9882 (1794)		--	--		2.51 (0.07)	11372 (4022)		2.55 (0.06)	11777 (3259)	d,
4	3.87 (0.04)	25721 (14735)		3.86 (0.03)	33571 (13207)	g,	3.90 (0.01)	35777 (19539)		3.90 (0.01)	31622 (11886)		3.90 (0.01)	23009 (9401)	g,
5-C1	--	--		5.96 (0.09)	109624 (136496)	e, f, g,	6.09 (0.07)	15356 (13469)	e,	6.11 (0.09)	15183 (8325)	f,	6.06 (0.09)	14263 (4506)	g,
6-C3	12.62 (0.08)	8601 (2233)	a, b, c, d,	12.62 (0.03)	18629 (7844)	a, f,	12.71 (0.03)	17975 (4401)	b, h,	12.69 (0.03)	30312 (12957)	c, f, h, j,	12.71 (0.03)	15104 (5906)	d, j,
7-C4	19.71 (0.07)	10925 (2735)	c, d,	19.75 (0.05)	11214 (1939)	f, g,	19.79 (0.09)	8924 (3672)		19.85 (0.07)	8271 (3269)	c, f,	19.87 (0.04)	7127 (1269)	d, g,
8	22.25 (0.07)	10726 (3997)	d,	22.27 (0.07)	11983 (4571)	g,	--	--		22.42 (0.08)	8181 (5616)		22.43 (0.08)	5748 (1357)	d, g,
9	23.56 (0.05)	8462 (1779)	b,	--	--		23.64 (0.03)	7310 (509)	b,	23.63 (0.04)	8170 (1540)		23.63 (0.04)	7857 (1097)	
10-C5	30.13 (0.04)	10292 (3091)	a, b, c,	30.11 (0.06)	18579 (7496)	a, g,	30.26 (0.04)	13955 (3882)	b, h, i,	30.25 (0.04)	23767 (13960)	c, h, j,	30.26 (0.04)	10047 (2748)	g, i, j,
11	--	--		31.59 (0.06)	7670 (1371)	e, g,	31.72 (0.04)	5233 (865)	e,	31.71 (0.05)	6102 (3247)		31.71 (0.03)	5124 (652)	g,
12	34.83 (0.04)	64362 (51817)	d,	34.84 (0.05)	35661 (16251)	g,	34.99 (0.04)	54571 (59109)		34.99 (0.04)	66984 (84158)		34.99 (0.03)	16696 (23832)	d, g,
13-C6	--	--		35.43 (0.06)	9945 (1756)	e, f, g,	35.59 (0.03)	6493 (772)	e,	35.59 (0.04)	7526 (2329)	f,	35.58 (0.04)	6829 (1452)	g,
14-C7	--	--		36.06 (0.11)	7882 (842)	e, f, g,	36.25 (0.05)	6463 (778)	e,	36.27 (0.05)	6569 (1613)	f,	36.24 (0.05)	5955 (528)	g,

15	46.14 (0.04)	10404 (2318)	c, d,	46.10 (0.07)	9922 (1506)	f, g,	46.30 (0.06)	8301 (3177)		46.31 (0.04)	8228 (1789)	c, f,	46.30 (0.04)	7760 (1206)	d, g,
16-C8	48.10 (0.08)	10895 (5148)		48.10 (0.09)	9703 (2285)	e, f, g,	48.16 (0.05)	13743 (6077)	e,	48.17 (0.07)	13705 (4113)	f,	48.15 (0.04)	13617 (3724)	g,
17	49.10 (0.05)	8023 (1342)	d,	49.09 (0.07)	7883 (1102)		49.30 (0.07)	7074 (1950)		--	--		49.30 (0.06)	6822 (1440)	d,
18	52.16 (0.05)	1266983 (572006)	b, c, d,	52.16 (0.06)	1197442 (519158)	e, f, g,	52.34 (0.04)	643679 (151311)	b, e,	52.34 (0.04)	694610 (163834)	c, f,	52.34 (0.04)	683378 (143723)	d, g,
19	--	--		53.07 (0.07)	7533 (822)	e, f, g,	53.24 (0.04)	6350 (650)	e,	53.25 (0.05)	6454 (1573)	f,	53.24 (0.04)	6179 (974)	g,
20	--	--		57.23 (0.07)	7272 (973)	f, g,	57.41 (0.04)	6267 (1843)		57.40 (0.05)	6073 (1417)	f,	57.39 (0.05)	6072 (1194)	g,
21-C9	67.17 (0.05)	22904 (3975)	b, c, d,	67.20 (0.06)	22780 (3323)	e, f, g,	67.34 (0.04)	14834 (4705)	b, e,	67.35 (0.04)	14875 (3667)	c, f,	67.33 (0.04)	13788 (2653)	d, g,
22-C10	68.59 (0.06)	18440 (4064)	b, c, d,	68.59 (0.06)	17319 (3374)	e, f, g,	68.76 (0.04)	11818 (3835)	b, e,	68.77 (0.05)	11820 (2142)	c, f,	68.76 (0.03)	11314 (2118)	d, g,
23	--	--		--	--		69.25 (0.05)	9487 (2036)	i,	69.24 (0.04)	10765 (4045)	j,	69.24 (0.04)	7184 (1790)	i, j,
24-C11	70.92 (0.06)	9138 (2726)	b,	70.92 (0.07)	8959 (1387)	e,	71.11 (0.03)	7200 (1726)	b, e,	71.12 (0.04)	7978 (1823)		71.11 (0.05)	7729 (1855)	
25	74.71 (0.06)	19220 (31982)		74.71 (0.07)	9778 (3313)	f,	74.90 (0.04)	47013 (141880)		74.89 (0.04)	6391 (1383)	f,	74.89 (0.04)	7673 (3784)	
26	75.99 (0.07)	8702 (1671)	c, d,	76.00 (0.06)	8063 (1127)	f, g,	76.17 (0.07)	7050 (3105)		76.18 (0.03)	6678 (1363)	c, f,	76.17 (0.04)	6539 (1047)	d, g,
27-C12	78.22 (0.07)	1330580 (594423)	b, c, d,	78.23 (0.07)	1231871 (331605)	e, f, g,	78.41 (0.03)	541882 (84018)	b, e,	78.41 (0.03)	607416 (206791)	c, f,	78.40 (0.03)	631543 (202320)	d, g,
28-C13	79.81 (0.06)	15112 (4098)	c, d,	79.82 (0.07)	13800 (2201)	f, g,	80.02 (0.04)	11344 (8837)		80.02 (0.03)	9559 (1669)	c, f,	80.00 (0.03)	9119 (1420)	d, g,
29-C14	88.63 (0.07)	15356 (23190)		88.61 (0.04)	7988 (2512)	e, f, g,	88.78 (0.14)	28348 (15442)	e,	88.82 (0.10)	23635 (13835)	f,	88.87 (0.04)	18743 (6348)	g,
30-C15	171.95 (0.02)	56526 (26770)		171.95 (0.02)	53134 (14408)	e, g,	172.01 (0.01)	39714 (10599)	e,	172.01 (0.01)	43781 (15802)		172.01 (0.01)	41137 (11452)	g,

Table 2: Peak distribution comparison in terms of retention time and peak amplitude for viral infected cell lines **(48 Hrs)**

['-C1, -C2, …': the first, second, … common peaks shared wit 24-hr infected cell lines. R.T.: retention time (min.). A.: amplitude (total ion counts, TIC), S.P.: separable peaks ($p<0.05$. Any two cell lines that have a same letter in their S.P. column are separable. '--': either no peak or not detected by the peak criteria. (*): standard deviation].

Peak No.	Cntrl			MOI 1 H1N1			MOI 10 H1N1			MOI 10 H6N2			MOI 10 H9N2		
	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.	R.T.	A.	S.P.
1-C1	1.94 (0.12)	25529 (51445)		2.06 (0.03)	18854 (5856)	e, f, g,	2.06 (0.01)	33738 (5590)	e, h, i,	2.04 (0.06)	53547 (18986)	f, h, j,	2.07 (0.01)	25269 (7255)	g, i, j,
2	3.86 (0.03)	40346 (20248)	d,	3.87 (0.03)	46689 (21178)	g,	3.95 (0.09)	34018 (34063)		3.94 (0.09)	29199 (40753)		3.94 (0.09)	18437 (8217)	d, g,
3-C2	5.97 (0.10)	102677 (139578)	b, c,	5.97 (0.09)	89269 (126787)	f,	6.14 (0.13)	15467 (13608)	b,	6.13 (0.07)	12979 (3486)	c, f,	--	--	
4	7.55 (0.09)	15842 (2333)	b, c,	7.54 (0.03)	15098 (2575)	e,	7.49 (0.12)	11645 (1463)	b, e,	7.53 (0.07)	12836 (3529)	c,	--	--	
5	10.21 (0.05)	15326 (2893)	b, d,	--	--		10.30 (0.03)	10372 (1276)	b,	10.25 (0.07)	12553 (4967)		10.29 (0.03)	10608 (1365)	d,
6-C3	12.61 (0.09)	24798 (55836)		12.63 (0.04)	19591 (5864)	g,	12.69 (0.04)	16392 (8453)		12.69 (0.04)	26769 (18189)	j,	12.71 (0.04)	13415 (6804)	g, j,
7	14.72 (0.06)	179950 (154023	c, d,	14.72 (0.04)	113587 (94050)	f, g,	--	--		14.82 (0.05)	26444 (23106)	c, f,	14.81 (0.04)	15569 (13826)	d, g,

		)													
8-C4	19.72 (0.09)	9553 (2108)	b, d,	--	--		19.84 (0.08)	7130 (1356)	b,	19.82 (0.08)	8032 (2811)		19.83 (0.08)	6812 (943)	d,
9-C5	30.14 (0.06)	22483 (45752)		30.13 (0.06)	16363 (5726)	e, g,	30.25 (0.03)	11215 (5801)	e,	30.24 (0.03)	13882 (8658)		30.24 (0.02)	9213 (3919)	g,
10-C6	35.41 (0.08)	9775 (2076)	b, c, d,	--	--		35.57 (0.03)	7075 (1161)	b,	35.57 (0.03)	7462 (2059)	c,	35.56 (0.02)	7940 (2096)	d,
11-C7	36.08 (0.09)	8018 (1601)	b,	--	--		36.23 (0.09)	6586 (1313)	b,	36.21 (0.10)	6805 (2256)		36.24 (0.02)	6810 (2098)	
12-C8	48.09 (0.06)	9725 (2024)	b, d,	48.11 (0.05)	10410 (2421)	e, g,	48.17 (0.08)	14765 (4105)	b, e,	48.17 (0.08)	30011 (55623)		48.16 (0.07)	14344 (3465)	d, g,
13	54.14 (0.11)	10403 (2693)	b, d,	--	--		54.18 (0.04)	13995 (2952)	b,	54.17 (0.04)	14774 (7340)		54.15 (0.04)	14641 (3457)	d,
14	59.29 (0.09)	111010 (222322)	d,	59.30 (0.06)	84668 (153458)	f, g,	59.36 (0.04)	168103 (201143)		59.35 (0.04)	302273 (255840)	f,	59.35 (0.03)	323340 (238440)	d, g,
15	64.02 (0.07)	9273 (1674)	b, c, d,	64.02 (0.07)	9604 (1234)	e, f, g,	64.17 (0.04)	11473 (1753)	b, e,	64.18 (0.04)	12581 (3356)	c, f,	64.17 (0.03)	11034 (1462)	d, g,
16-C9	67.18 (0.06)	19993 (3883)	b, c, d,	67.18 (0.06)	20658 (3291)	e, f, g,	67.31 (0.03)	12935 (1536)	b, e,	67.31 (0.04)	14845 (2888)	c, f,	67.30 (0.02)	14429 (2331)	d, g,
17-C10	68.59 (0.07)	15346 (4069)	b, d,	68.60 (0.06)	16455 (2832)	e, f, g,	68.73 (0.05)	11029 (1260)	b, e, h,	68.73 (0.03)	12784 (2488)	f, h,	68.73 (0.02)	12172 (1807)	d, g,
18-C11	70.89 (0.09)	8421 (1189)		70.91 (0.07)	8850 (1185)	e,	71.08 (0.04)	7600 (1047)	e,	71.08 (0.03)	31796 (75434)		71.08 (0.03)	62545 (127604)	
19	71.91 (0.11)	11507 (4414)	b, c, d,	--	--		72.02 (0.03)	19162 (7885)	b,	72.02 (0.04)	25760 (16566)	c,	72.01 (0.02)	23731 (10620)	d,
20	73.86 (0.08)	11168 (3156)	b, d,	73.88 (0.06)	11930 (3214)	e, g,	74.00 (0.04)	17519 (4569)	b, e,	74.00 (0.04)	42791 (84895)		73.99 (0.03)	17945 (4636)	d, g,
21-C12	78.23 (0.07)	1082191 (550038)	b, d,	78.23 (0.07)	1100144 (339174)	e, f, g,	78.39 (0.03)	704250 (290248)	b, e,	78.39 (0.03)	741078 (362434)	f,	78.38 (0.02)	615519 (79339)	d, g,
22-C13	79.81 (0.07)	12586 (2391)	b, d,	79.82 (0.07)	13369 (2263)	e, g,	79.99 (0.04)	9402 (1136)	b, e,	79.99 (0.03)	11363 (3899)		79.97 (0.02)	10182 (1530)	d, g,
23	82.45 (0.08)	9234 (1691)	b, d,	82.44 (0.08)	9489 (1650)	e, g,	82.65 (0.04)	6864 (811)	b, e,	82.63 (0.04)	8171 (2632)		82.62 (0.03)	7477 (1220)	d, g,
24	87.42 (0.10)	9159 (2849)	b,	87.42 (0.10)	10775 (2610)	e,	87.22 (0.04)	5061 (776)	b, e,	--	--		--	--	
25-C14	88.65 (0.09)	7924 (3340)	d,	88.64 (0.09)	9122 (4012)	g,	88.82 (0.07)	36445 (52712)		88.82 (0.08)	38360 (73221)		88.83 (0.02)	13851 (2398)	d, g,
26	93.74 (0.09)	8192 (1270)		93.75 (0.09)	8483 (966)	e,	93.95 (0.03)	7494 (853)	e,	93.94 (0.04)	8659 (3278)		93.93 (0.02)	7714 (1267)	
27	98.04 (0.11)	7070 (745)	d,	98.07 (0.09)	7221 (737)	g,	98.25 (0.03)	7732 (1315)		98.25 (0.04)	10464 (7911)		98.24 (0.03)	8228 (1482)	d, g,
28	99.32 (0.11)	12242 (1900)	b,	99.34 (0.09)	12497 (1622)	e, g,	99.53 (0.03)	10503 (1515)	b, e,	99.53 (0.03)	12592 (5548)		99.51 (0.03)	10932 (1428)	g,
29	103.62 (0.10)	8448 (1468)	b, d,	103.64 (0.09)	8617 (1278)	e, g,	103.82 (0.03)	6849 (949)	b, e,	103.82 (0.03)	8540 (3389)		103.81 (0.03)	7038 (1340)	d, g,
30	105.23 (0.10)	805694 (337260)	b, c, d,	105.21 (0.09)	768191 (383879)	g,	105.44 (0.03)	503925 (281715)	b,	105.43 (0.03)	512657 (311542)	c,	105.43 (0.02)	378100 (96344)	d, g,
31	109.99 (0.10)	745719 (498143)	b, c, d,	110.01 (0.08)	764123 (519828)	e, f, g,	110.21 (0.04)	82253 (80126)	b, e,	110.20 (0.03)	91151 (69804)	c, f,	110.19 (0.02)	100964 (63629)	d, g,
32	160.54 (0.08)	8393 (2970)	b, c, d,	160.56 (0.08)	8994 (3189)	f, g,	160.44 (0.04)	11476 (3815)	b,	160.43 (0.04)	15905 (6750)	c, f,	160.43 (0.03)	13941 (4602)	d, g,
33-C15	171.95 (0.03)	50276 (14640)	b,	171.95 (0.02)	53664 (13519)	e, g,	172.01 (0.01)	38542 (10354)	b, e,	172.01 (0.01)	58276 (46680)		172.00 (0.01)	41282 (11015)	g,
34	--	--		--	--		173.27 (0.03)	24008 (5060)	h,	173.24 (0.08)	30585 (9475)	h,	173.28 (0.04)	27760 (5113)	

Table 3: Chemical ID and match factors for the common peaks of both 24hr and 48hr viral infected cell lines (N.D.: no dominant chemical with high match factor)

Peak No.	Chem ID	Cntrl		MOI 1 H1N1		MOI 10 H1N1		MOI 10 H6N2		MOI 10 H9N2	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
	(S)-2-Hydroxypropanoic acid	93	72	85	89	78	84	80	81	84	86
C1	Propanoic acid, 2-hydroxy-, ethyl ester	91	73	80	87	79	80			80	81
	Butanoic acid, anhydride	--	77	76	78	74	75	73	76		--
C2	Furan, tetrahydro-		73	73	74	75	74	78	74	86	
C3	Propanoic acid, ethyl ester	80	87	85	83	86	85	86	88	87	86
	Butanoic acid, 2-methyl-, methyl ester	89	85	90	--	84	87	88	87	90	84
C4	Methyl 2-methylhexanoate	69	73	78		67	73	71	73	76	70
C5	Butanoic acid, 2-methyl-, ethyl ester	81	90	87	87	90	88	73	89	90	88
C6	3-Hexanone, 5-methyl-	--	84	85	--	82	88	87	92	88	88
	2-Heptanone	--	80	85	--	80	75	76	82	76	77
C7	2-Octanone		62	72		69	75	66	71	70	71
	Phenyl-pentamethyl-disiloxane	61	52	56	57	58	58	57	61	61	60
	Benzoic acid, 2-formyl-4,6-dimethoxy-	58	55	54	58	55	60	62	61	61	61
C8	, 8,8-dimethoxyoct-2-yl ester										
	Benzene, 1-ethenyl-3-ethyl-	93	86	93	93	92	88	90	93	92	90
C9	Benzene, 1-ethenyl-4-ethyl-	92	83	92	92	90	87	90	91	90	89
	Benzene, 1-ethenyl-4-ethyl-	91	94	87	89	88	89	90	90	89	88
C10	Benzene, 1-ethenyl-3-ethyl-	89	91	87	87	87	89	87	89	87	87
	Decane, 2,4-dimethyl-	78	64		70	73	73			73	73.
	Nonane, 3,7-dimethyl-	76	66	72	72	73	74		72	73	75
C11	Butane, 2,2-dimethyl-	86		83	74	73	73	70			74
C12		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Benzaldehyde, 4-ethyl-	91	90	89	90	90	89	89	88	90	90
C13	Benzaldehyde, 3-ethyl-	85	85	85	85	88	85	85	86	85	84
	Cyclohexanepropanol-	67	65	72	57						
	Cyclodecanol					81	85	84	84	67	84
	Bicyclo[4.1.0]heptane, 7-pentyl-	63	59		60						
C14	Z-4-Dodecenol	68	59	72	76					68	83
C15	- siloxane	51	57	58	51	47	51	49	55	58	74