A BREATHPRINT OF INFLUENZA A VIRUS INFECTION IN THE FERRET MODEL

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INTRODUCTION

Influenza viruses (IV) pose a major public health concern, since they are highly contagious and still have a high global annual mortality. Current diagnostic methods are slow or have high error rates; therefore, a rapid detection tool is highly desirable to inform clinical management. Exhaled breath is a useful diagnostic in a number of diseases. In this work, we examined the exhaled breath of six ferrets collected pre- and post-IV infection. Exhaled breath analysis was carried out using a comprehensive two-dimensional gas chromatography (GC × GC) hyphenated with a time-of-flight mass spectrometer (ToF MS). Random Forest, a non-parametric machine learning algorithm, was used to select the most relevant diagnostic volatile organic compound (VOC) features and build the model using a cross-validation approach. Using this approach, we defined 31 VOC features, which together produce a profile capable of discriminating between uninfected and IV-infected ferrets. Further characterization revealed an abundance of hydrocarbons, which is consistent with increased oxidative stress known to occur during viral infection. Our study is the first of its kind to define a unique exhaled breath signature for influenza infection in ferrets and will be useful for development of a rapid precision diagnostic technique.

EXPERIMENTAL

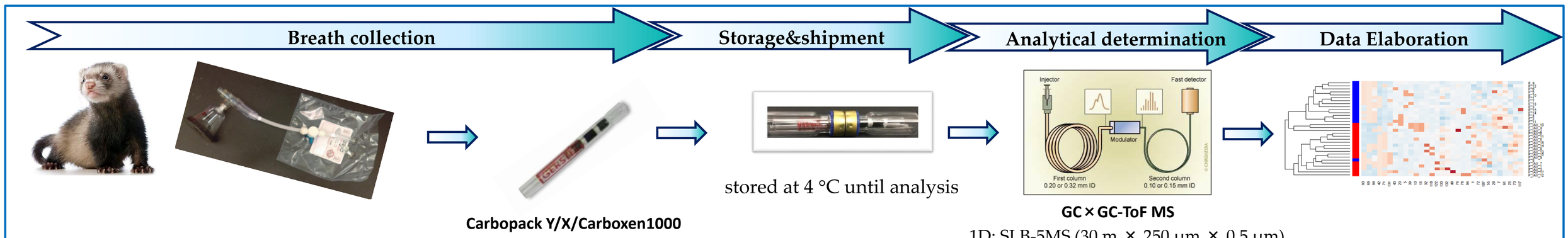
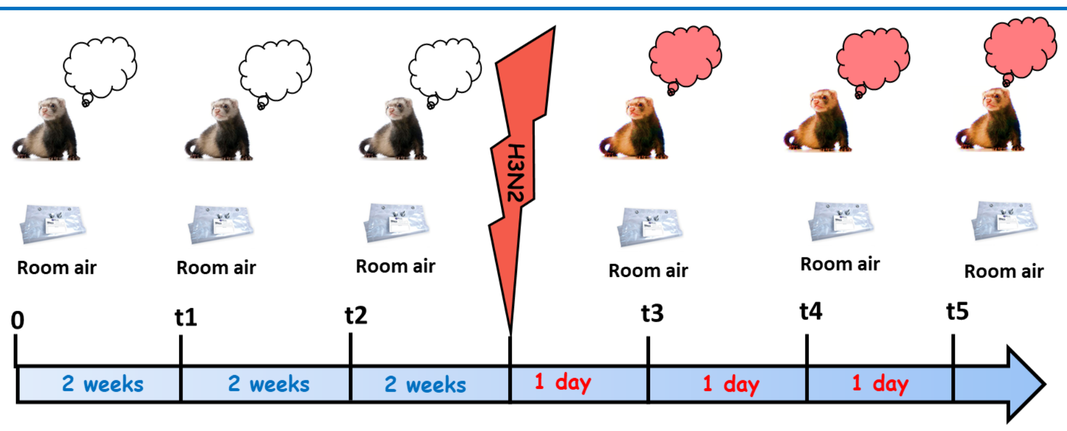


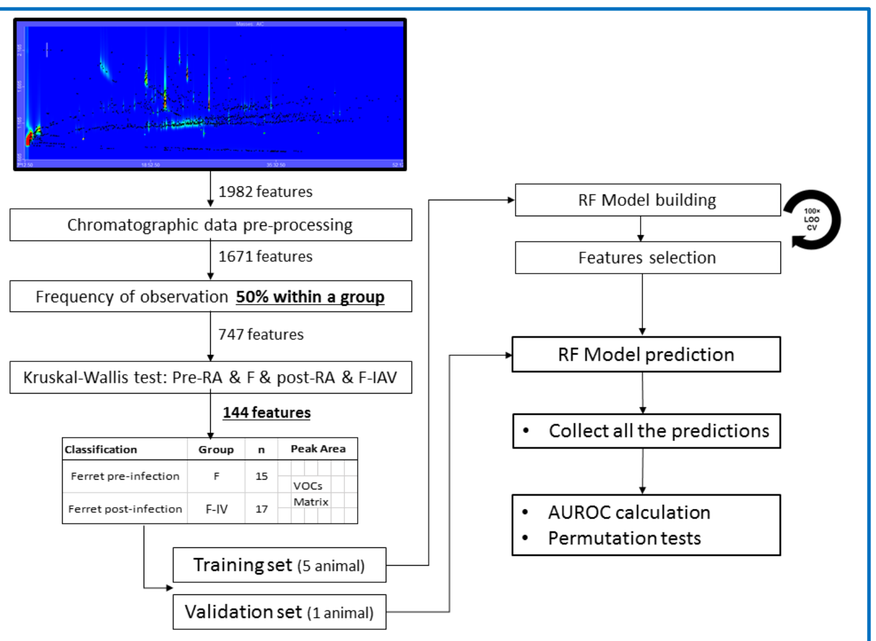
Figure 1. Overall analytical procedure.



Class	t0	t1	t2	t3	t4	t5	Total
F	6	6	6				18
F-IV				6	6	6	18
RA	3	3	3	3	3	3	18

Figure 2. Experimental design. Influenza Virus: H3N2 A/Perth/16/2009 (predominant strain in 2016-2017 and 2017-2018 seasons). F: Pre infection ferret; F-IV: Post-infection.

Figure 3. Data cleaning and processing scheme. Pre-RA: Room air sampled before infection; post-RA: Room air sampled after infection; F: breath samples from ferret pre-infection; F-IV: breath samples collected from ferret infected with influenza A virus (IV). RF: Random Forest; AUROC: area under the receiver operating characteristic curve.



RESULTS AND DISCUSSION

To discriminate between infected and uninfected animals, volatile features were selected using the Random forest (RF) classification algorithm using a leaving-one-animal out approach. At each iteration the features were ranked according to their mean decrease in accuracy and the features present in the top 15 at least 50% of the time were retained as the most discriminatory of IV infection. A total of 31 features (Table 1) were selected and used to build the final model using the samples in the validation set. The result was visualized using heatmap and hierarchical clustering analysis (Figure 4). The performance of the model was visualized by generating a receiver operating characteristic (ROC) curve using the aggregate validation set class probabilities (ranging from 0 to 1) for each animal (Figure 5).

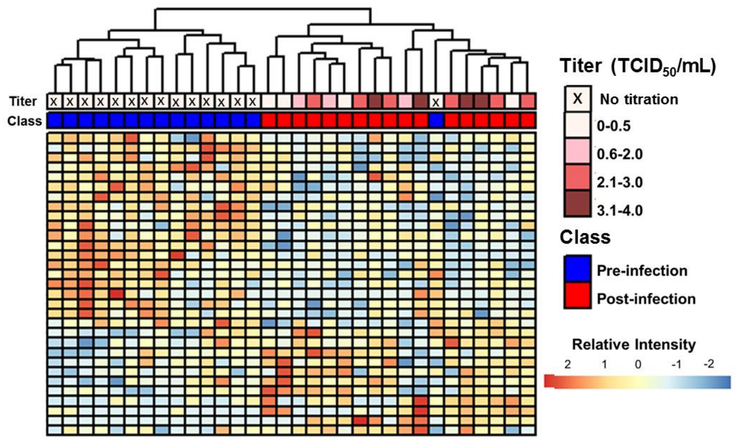


Figure 4. Heatmap depicting the relative abundance of the 31 selected VOCs as a function of sample. The virus burden determined as tissue culture infectious dose 50 (TCID50) per mL from nasal wash titers. The color key is based on the peak intensity after auto-scaling per variable.

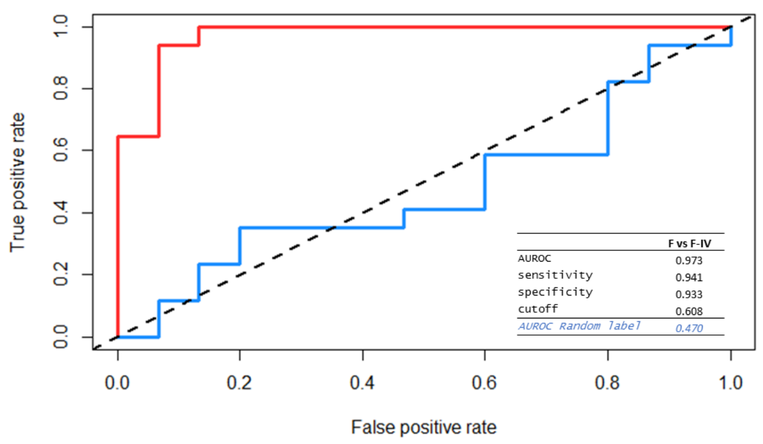


Figure 5. Aggregate ROC curve for the discrimination between F versus F-IV, generated using Random Forest on the 31 selected features (red line). Moreover, ROC curve obtained from a label permutation test is reported (blue line).

Table 1. List of the volatile metabolites selected as the most discriminatory for determining animal infected with IV. 10 VOCs were identified at level 1 according to the criteria established by the Metabolomics Standards Initiative (MSI) [1].

#ID	Compound	Chemical class*	CAS	MS%	LRI Exp	LRI Lit	1D rT (min:sec)	2D rT (sec)
1	3-methylene-heptane	Hyd	1632-16-2		789	793	9:00	1.1
2	alkylate hydrocarbon	Hyd			844		11:25	1.1
3	alkylate hydrocarbon	Hyd			855		11:55	1.1
4	alkylate hydrocarbon	Hyd			877		12:56	1.1
5	alkylate hydrocarbon	Hyd			881		13:09	1.2
6	Cyclohexanone	Ket	108-94-1	855	896	901	13:50	1.8
7	Ketone	Ket			907		14:21	1.3
8	Alkylate hydrocarbon	Hyd			910		14:30	1.1
9	Aldehyde	Ald			922		15:08	1.3
10	Alkylate hydrocarbon	Hyd			949		16:33	1.1
11	2-ethyl-hexanal	Ald	123-05-7	919	954	952	16:47	1.4
12	1-Heptanol	Alc	111-70-6	800	969	970	17:34	1.4
13	Alkylate hydrocarbon	Hyd			975		17:53	1.1
14	Octanal	Ald	124-13-0	892	1003	1006	19:20	1.5
15	Alkylate hydrocarbon	Hyd			1023		20:20	1.3
16	Unknown	Unk			1047		21:34	1.5
17	Unknown	Unk			1059		22:14	1.6
18	Phenyl acetate	Ace	122-79-2	824	1058	1068	22:10	1.9
19	2(E)-octenal	Ald	2548-87-0	822	1060	1059	22:15	1.6
20	Acetophenone	Ket	98-86-2	930	1069	1068	22:42	2.1
21	Alcohol	Alc			1078		23:12	1.4
22	Alkylate hydrocarbon	Hyd			1152		26:55:00	1.2
23	isododecane isomer (2-methyl-undecane)	Hyd	7045-71-8	878	1164	1164	27:30:00	1.2
24	Alkylate hydrocarbon	Hyd			1180		28:18:00	1.2
25	Decanal	Ald	112-31-2	866	1207	1208	29:35:00	1.5
26	Benzothiazole	Aro	95-16-9	809	1233	1234	30:50:00	2.4
27	Unknown	Unk			1273		32:43:00	2
28	Unknown	Unk			1302		34:03:00	1.8
29	Alkylate hydrocarbon	Hyd			1446		40:28:00	1.2
30	Ketone	Ket			1446		40:28:00	1.6
31	Alkylate hydrocarbon	Hyd			1485		41:59:00	1.2

** Hyd: hydrocarbon; Ald: aldehyde; Ket: ketone; Alc: alcohol; Ace: acetate; Aro: aromatic.*

CONCLUSIONS

Our study represents the first comprehensive evaluation of the VOCs present in breath of virally infected animals. Although more work needs to be done to understand the metabolic origins for the identified VOCs, our study provides the first important step in defining an exhaled breath biomarker suite for influenza infection.

[1] Sumner et al., Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI), Metabolomics 3, 211–221 (2007).

Breath Biopsy Conference, Cambridge Science Park, Cambridge UK, November 08, 2018