Virus Detection Dogs: Proof of Concept Study

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Canine Performance Sciences

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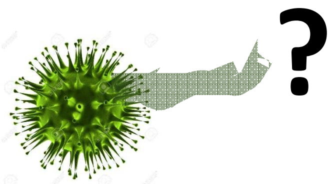
Outline

- Use the literature to answer fundamental questions
- Project Objectives
- Methods
- Results
- Conclusions
- Future Research



Do pathogens and diseases produce an odor?

- Shirasu and Kazushige (2011) stated if a biological system contracts an infection or metabolic disease, then unique VOC patterns can be released and detected as an odor
- The study cited over 50 articles that described the odor certain diseases and disorders produce

J. Biochem. 2011;150(3):257–266 doi:10.1093/jb/mvr090
JB THE JOURNAL OF BIOCHEMISTRY

JB Review

The scent of disease: volatile organic compounds of the human body related to disease and disorder

Received May 27, 2011; accepted July 6, 2011; published online July 19, 2011

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Hundreds of volatile organic compounds (VOCs) are emitted from the human body, and the components of

Human body odours also have this function; we emit a wide array of volatile organic compounds (VOCs), both odorous and non-odorous, from our bodies. The VOCs emitted from different areas of the human body vary with age, diet, sex, physiological status and possibly genetic background. Therefore, body odours can be considered as individual 'odour-fingerprints'.

Pathological processes, such as infection and endogenous metabolic disorders, can influence our daily odour fingerprints by producing new VOCs or by changing the ratio of VOCs that are produced normally.

Are there unique viral VOC's?

- Aksenov et al. (2014) found unique VOC patterns in response of infection to B lymphoblastoid cells with three live influenza virus subtypes: H9N2 (avian), H6N2 (avian), and H1N1 (human).
- Schivo et al (2014) found that there are different expressions of VOCs between uninfected and human rhinovirus infected bronchial epithelial cells.
- Mashir et al. (2011) administered live attenuated H1N1 vaccine (FluMist®) to humans and found exhaled breath volatiles increased for seven days after the vaccination.

CHEMBIOCHEM
FULL PAPERS
European Journal of Chemical Biology, Synthetic Biology, and Bio-Nanotechnology
ChemPubSoc Europe

DOI: 10.1002/cbic.201300695

Cellular Scent of Influenza Virus Infection.

Alexander A. Aksenov,[a] Christian E. Sandrock,[b] Weixiang Zhao,[a] Shankar Sankaran,[a] Michael Schivo,[b] Richart Harper,[b] Carol J. Cardona,[c] Zheng Xing,[c, d] and Cristina E. Davis*[a]

Volatile organic compounds (VOCs) emanating from humans have the potential to revolutionize non-invasive diagnostics. Yet, little is known about how these compounds are generated by complex biological systems, and even less is known about how these compounds are reflective of a particular physiological state. In this proof-of-concept study, we examined VOCs produced directly at the cellular level from B lymphoblastoid cells upon infection with three live influenza virus subtypes: H9N2 (avian), H6N2 (avian), and H1N1 (human). Using a single

duction upon infection of the cells. The patterns of VOCs produced in response to infection were unique for each virus subtype, while several other non-specific VOCs were produced after infections with all three strains. Also, there was a specific time course of VOC release post infection. Among emitted VOCs, production of esters and other oxygenated compounds was particularly notable, and these may be attributed to increased oxidative stress resulting from infection. Elucidating VOC signatures that result from the host cells response to in-

Can VOCs be detected outside the body for non-invasive sampling?

- Simultaneous measurements of blood and breathborne VOCs were performed in healthy volunteers, enabling endogenous compounds to be distinguished from exogenous compounds (Mochalski et al. 2013)
- Amann et al. (2014) cited multiple studies that measured VOCs outside the the body in exhaled breath, skin, urine, feces, and saliva.

Topical Review

Journal of Breath Research (2014)

The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva

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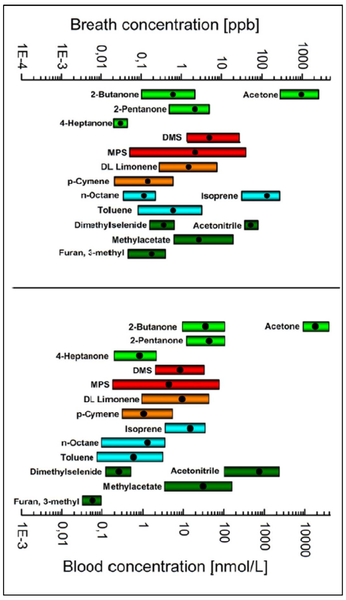


What detection tools are sensitive enough to detect VOC's?

MOBILE VOC Detection Technology

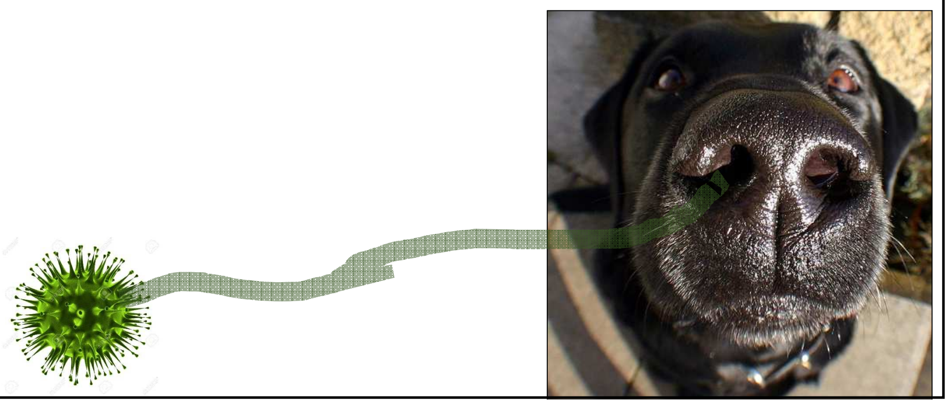
Lab Based VOC Detection Technology

Are canines capable of detecting pathogens or diseased tissue?

	Sensitivity	Specificity
Breath Samples • Sonoda et al. (2011) • McCulloch et al., (2006)	91% (Colon) 99% (Lung) 88% (Breast)	99% 99% 98%
Stool Samples • Bomers et al. (2012) • Durgin et al. (2012) (Rat) • Sonoda et al. (2011)	83% (*C difficile*) 100% (*Salmonella*) 97% (Colon)	98% 96% 99%
Urine Samples • Willis et al. (2004) • Cornu et. al., (2010)	41% (Bladder) 91% (Prostate)	N/A 91%
Sputum Sample • Mgode et al. (2012) (Rat)	80.4% (*Mycobacterium Tuberculosis)*	72.4%
Skin Lesions • Pickel et al. (2004)	75-85.7% *(Melanoma)*	N/A
Tissue Samples • Horvath et al. (2008)	100% *(Ovarian)*	97.5%

Literature Summary

- Pathogens do have an odor
- There are unique VOC's associated with viruses
- The VOCs can be detected outside the body
- Canines can detect pathogens and diseases



Pilot Project Objectives

Objective:

- Train dogs to provide real time detection of lab grown Bovine Viral Diarrhea Virus (BVDV)
- Train dogs to discriminate a target virus from other distractor viruses (BHV and PI3)
- Determine the sensitivity and specificity of real time virus detection

Methods: Targets and Distractors

Samples contained 5 x 10^4 to 5 x 10^5 $CCID_{50}$ (cell culture infective doses, 50% endpoint) Bovine Viral Diarrhea Virus per 0.5 milliliter of media.

Targets
1A AU526 + MDBK + **EQS** **(Noncytopathic)**
2A AU526 + MDBK + **FBS** **(Noncytopathic)**
3A AU526 + MDBK + **Gentamicin** **(Noncytopathic)**
4A NADL + MDBK + **EQS** **(Cytopathic)**
5A NADL + MDBK + **FBS** **(Cytopathic)**
6A NADL + MDBK + **Gentamicin** **(Cytopathic)**

Distractors
1B = MDBK + **EQS**
2B = MDBK + **FBS**
3B = MDBK + **Gentamicin**
7A = **BHV-1** + MDBK + **EQS** **(Cytopathic)**
8A = **BHV-1** + MDBK + **FBS** **(Cytopathic)**
9A = **BHV-1** + MDBK + **Gentamicin** **(Cytopathic)**
10A = **PI-3** + MDBK + **EQS** **(Cytopathic)**
11A = **PI-3** + MDBK + **FBS** **(Cytopathic)**
12A = **PI-3** + MDBK + **Gentamicin** **(Cytopathic)**

Methods

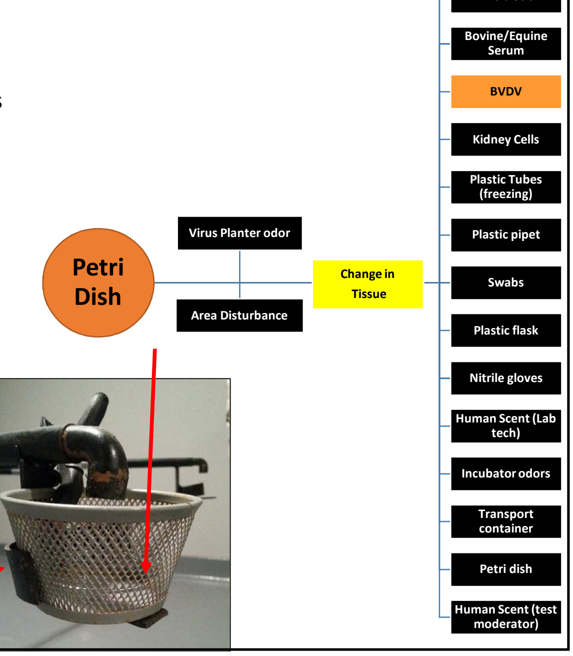
Testing Procedures

- An 8 arm scent wheel was used
- The same person put out all the positive and distractor samples on the scent wheel
- Target position was randomized on the scent wheel
- 1 trial was equal to one time around the eight positions, then the dog was called out of the room
- All 8 Baskets are changed out every single trial (no basket is used twice to prevent marking)
- The dog was off lead
- The handler was blind

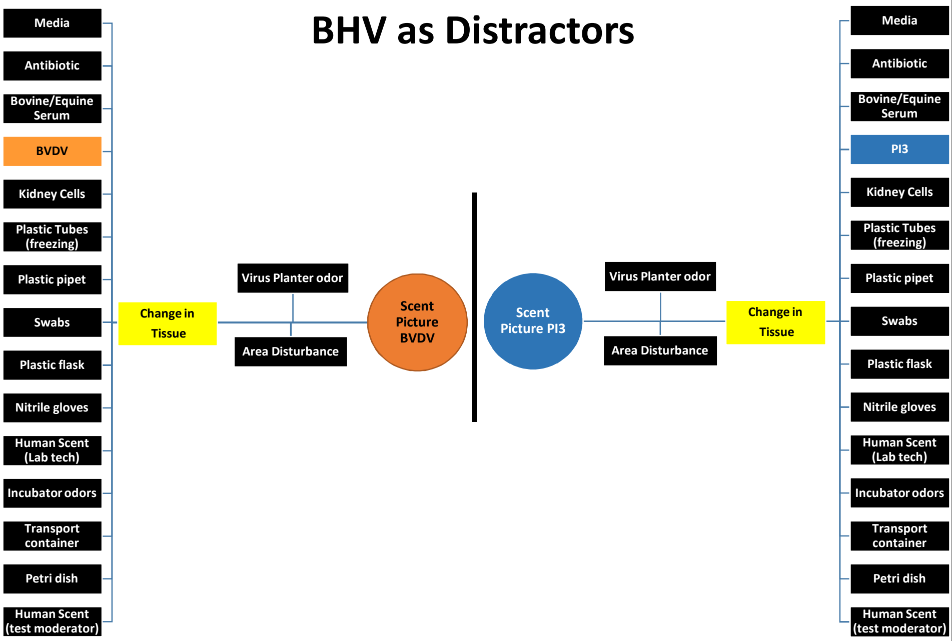
Target Scent Picture

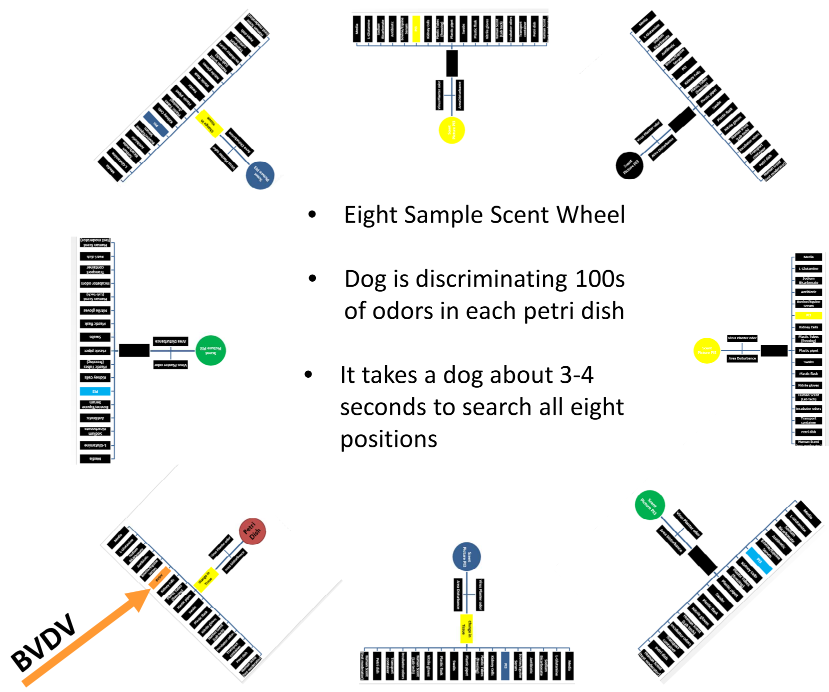
Petri Dish

- The petri dish contains 100+ odors
- We had to train the dog to only search for BVDV



Introduction of PI3 and BHV as Distractors





- Eight Sample Scent Wheel
- Dog is discriminating 100s of odors in each petri dish
- It takes a dog about 3-4 seconds to search all eight positions

Results of Pilot Data

Dogs are able to distinguish samples containing 5 x 10^4 to 5 x 10^5 $CCID_{50}$ (cell culture infective doses, 50% endpoint) Bovine Viral Diarrhea Virus per 0.5 milliliter of media from other virus distractor samples.

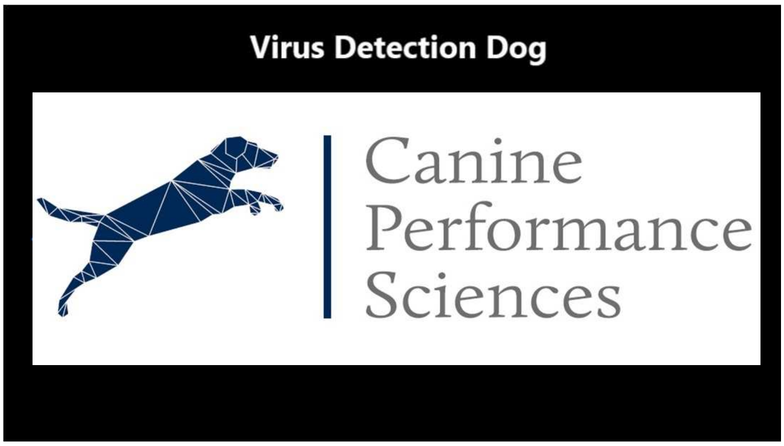
	Baxter	Moose
Sensitivity	**82.3**	**96.7**
Specificity	**98.1**	**99.3**
Total Number of Positive Trials	**34**	**31**
Total Number of Blank Trials (i.e. no BVDV present)	**24**	**20**
Total Number of Negative Samples Searched	**317**	**287**
Total Number of False Negative Indications	**6**	**1**
Total Number of False Positive Indications	**6**	**2**

Note: There was a low number of study trials (i.e. 109) but the dogs discriminated a large number of samples (i.e. 604)

Conclusions

- Canines are capable of identifying a target virus
- Canines are capable of discriminating a virus from other viruses
- Canines can provide a high rate of sensitivity and specificity.
- More robust research needs to be conducted
  - double blinded, multiple targets, operationally relevant experiments

Developing the Capability



Questions?

Canine
Performance
Sciences

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