Evaluation of an Ion Capture Method for Determination of Aerosolized Venezuelan Equine Encaphlitis Virus and a Novel Method for Absolute Particle Count Determination.

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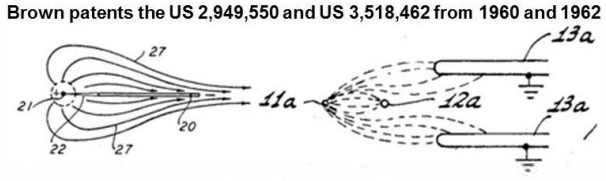
Abstract

Ionic capture devices have had widespread use for air cleaning_ We are exploring the use of a miniature device (clCD) for capture and detection of allergens, bacteria, viruses and toxins. Here we present data for its use in detection of gamma-irradiated inactivated Venezuelan equine encephalitis virus. Samples are collected on removable silk envelopes covering the entire electrodes. As references, data was obtained by extraction from the electrodes directly, and with a sampling fitter run in parallel. Capture was quantitated by q-RT-PCR. Controlled aerosols were released into an environmental chamber and particle size and concentration were held constant during 30 minutes of sampling. There is a possibility that irradiation damage to the viral RNA rendered it un-amplifiable. An alternative method of evaluation was devised based on limiting dilution condition where amplification occurred in some, but not all, RTPCR reactions. A discrete number of amplifiable particles per sample is present, and the amplification is a stochastic event determined by a Poisson distribution. We make the approximation that there is, on average, only one amplifiable molecule per tube when the number of amplifiable tubes is less than 50%. From that, the average number of amplifiable molecules is calculated. Using this metric, the LOO for the sampler is 0.06 amplifiable particles per liter and for the reference filter it is 1.4. The higher LOO for the reference filter is simply accounted for by the fact that the extraction is done in 10 times the volume compared with the electrodes. Capture efficiencies and LOD's from the disposable and the entire electrode were equivalent. The number of amplifiable particles is considerably lower than the number calculated from the calibration based on the original PFU. The stochastic method provides a method for determining an absolute amplifiable particle count, independent of any calibration.

Background

Aerosol sampling devices have been available for many years based on the principles of impacting a solid or gel surface, impinging a liquid surface or filtration through microfilters. All of these methods require pumping air through against some resistive forces. The methods thererefore require significant power, are noisy and require skilled handling. Custis et al (Clin Exp Allergy 2003, **33,** 986-991) introduced the use of an air cleaning device with no moving parts to collect allergens in dust particles. We have been exploiting the use of a miniature version of the same device for collection of a variety of aerosol particles, including common household allergens and bacteria such as Mycobacterium tuberculosis. Here we extend this to the demonstration of capture of virus particles and some interesting consequences of using PCR to measure the captured virus at low concentrations.

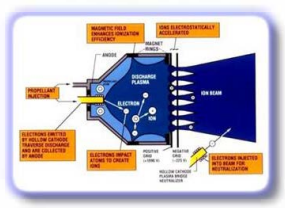
Brown patents the US 2,949,550 and US 3,518,462 from 1960 and 1962



He patented the principle of electrokinetic propulsion:
"An electrokinetic apparatus for utilizing electrical potentials for the production of forces for the purpose of causing relative motion between a structure and the surrounding medium". A wire at high potential creates a high voltage gradient, creating a local plasma which imparts charge on particles, which are then propelled to oppositely charged plate electrodes.

The same principle has been used for

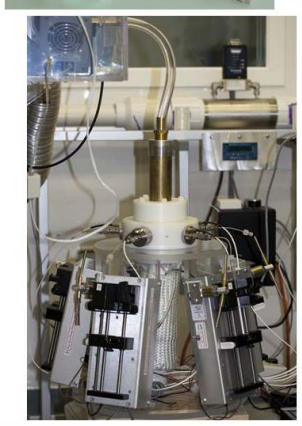
Interplanetary transport and air cleaning



http://www.grc.nasa.gov/WWW/ion/overview/overview.htm

Methods

A removable electrode assembly which captures particles is shown. The electrodes are covered with silk envelopes and the device is plugged in. Al the end of a run, the envelopes are removed and placed in Falcon tubes. One m l of double distilled water was added and the tubes vortexed intermittently over 10 minutes. Aliquots are added to the PCR reactions. Ih some experiments the electrodes were extracted directly in 50 m l Falcon tubes.



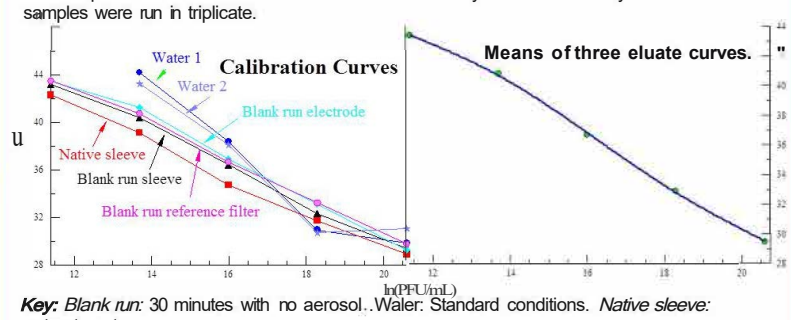
Sono-Tek120 kHz piezoelectric-driven atomizer fed by a syringe pump produces particles by generating -30µm droplets. The droplets are transported into a column of healed rising air. The sizes of the residual particles are directly related to the concentration of material in solution.

The aerosol is transported into the chamber and up to three clCOs are run in the chamber in parallel, together with 47 mm polycarbonate filters of 0.8 µm pore size.

The Venezuelan Equine Encaphilitis virus was from ECBC stocks that have been extensively gamma-irradiated to permit use in a BSL-2 facility.

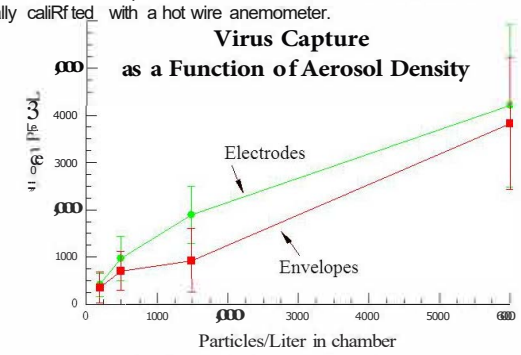
Results

Calibration: Possible effects of extractables on the PCR were investigated with 30 min runs with no aerosol and spiking with dilutions of the standard stock of virus solution. No amplification was obtained with no added virus. Viral RNA amplification was with an Applied Biosystems 7500 FastOx Real-Time PCR System. 5µl of sample is used in each well with 14.6ul master mix and 0.4ul Taq. Threshold was setto 0.05 and baseline start cycle= 3 and end cycle= 15. All samples were run in triplicate.



Key: *Blank run:* 30 minutes with no aerosol..Waler: Standard conditions. *Native sleeve:* no treatment.
Conclusion: The addition of a mock sample affects the calibration in a subtle way. However, there was no significan difference within mock samples. Therefore, the means from these three runs were re-plotted and the cubic polynomial fit used for and used to transform Ct values:
CFUlmL = e(152-10.3Ct+0.27Ct2-0.0025Ct3)

Capture on sleeves v. capture on electrode. The aim is verify whether the silk envelopes capture the particles efficiently or whether a significant fraction is lost. The aerosol density was successively reduced amount captured determined. Bars represent ±standard deviations from 18 data points for electrodes, 36 for the envelopes. The PFU/L of air was calculated from the known volume of air sampled in 30 minute runs, based on volume flow for each clCO individually caliRf ted with a hot wire anemometer.



Conclusion. The amount collected by the envelopes is less than on the electrodes, but the difference is borde ine significant.

Limits of Detection. The limits of detection (LOO) were determined by the "classical method" as 2X the standard deviation at a level approximating to the limit of detection. The 100 particle per liter values were such that less than 50% of the individual PCR reactions were amplified. We define an "amplifiable unit" (AU) as an individual amplifiable particle. From the Poisson distribution, each reaction that amplifies contains approximately one AU. Thus, a simple count of number of assays yielding amplifiable product represents an alternative method of measuring an LOO. Both methods of calculation are shown. The values or from18 data points for electrodes, 36 for the envelopes, 9 for the membranes.

	LOD eer liter	
Caeture b	PFU	AU
Electrode	288	0.05
Sleeve	354	0.04
Membrane	2052	0.91

Conclusion. The number of AU is vastly lower than the PFU. The PFU is based on the original titer prior to gamma-irradiation. Thus, the gamma-irradiation not only inactivates the virus, but reduces the amplifiability by 4 orders of magnitude. The LOO values for the cl CO are systematically lower than those of the reference membrane filter since the filter air flow is approximately 1/3 of that of the clCO and the ECBC standard extraction method is in 10 m l compared with 1 m l for the clCO.

Effect of clCD Plasma on Amplifiability: The use of gamma-irradiated virus stocks showed that the irradiation resulted in a loss of amplifiablity.. The cl CO exposes the aerosol to ionizing plasma as part of the capture process. Il is possible that this exposure will also reduce the amplifiability of individual particles. An additional metric for the effect of the irradiation on the AU is damage to genome structure reducing the amplifiability of individual particles. This would result in an increase in the Ct values. The mean Ct values for those reactions which amplified are shown

	Number of sameles		Ct
Caetured on	Amelified	Non-amelified	Mean+ SD
Electrodes	8	10	42.4± 1.0
Sleeves	10	26	42.6 ± 0.8
Reference	3	6	43.1 + 0.3

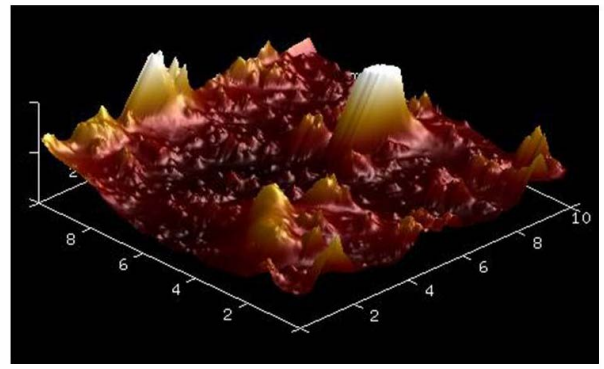
Conclusion. There appears to be no significant difference in the amplifiablity of AUs captured by the cl CO orby the reference filter. The reference filter involves no irradiation.

Effect of Aerosol Particle Size: Since the particle trajectories in the clCO depend largely on charge and not on mass, there is no obvious lower limit to the size of particle that can be captured. As smaller aerosol particles are generated, the signal per particle decreases. Therefore, compensatory increases in particle concentration must be made in order to obtain a sufficient signal. Successive 30 minute sampling runs were therefore made according to the schedule of the following table. Capture efficiencies were determined from the computed number of particles per liter of air from the clCO, relative to the same calculated values from the reference fillers, and expressed as percent. All values were from direct extraction from electrodes.

Input Particles		% Capture efficienc:t	
Mean size (micron)	Concentration (eer liter)	Mean	±SD
3.4	100	41	24
3.2	250	49	31
3.5	300	105	38
3.6	500	23	9
3.2	750	48	27
3.3	1000	19	9
3.2	3000	41	22
3.6	6000	38	16
3.2	9000	34	6
1.64	4500	41	15
1.64	8000	18	8
1.64	11000	25	7
1	30000	26	7

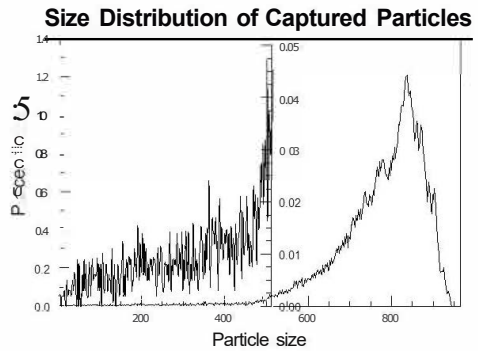
Conclusions. Capture efficiency was maintained going down to the lowest particle size tested. The range was limited by the intrinsic capabilities of the system and analytical methods.

Lower range of particle sizes: Since smaller aerosol particle sizes remain suspended longer, there may be a population of particles in the air going down to the nanometer range which would normally escape notice. We there used Atomic Force Microscopy (AFM) to determine whether such particles indeed exist. The clCO was run for 6 days in a clean domestic environment. A visible film was discernible on the electrodes. This was examined with a Bruker dimension ICON system which has the capability of providing sub-nm resolution. Imaging was done in tapping mode with super sharp silicon probes. Results were analyzed with the Bruker Analysis software.



Three-dimensional representation of paticles captured on clCO. Scales are in nanometers

Size Distribution of Captured Particles



Histogram representation of particle size distribution captured on cCO electrode. Inset is on a 20X expanded scale to reveal the lower end of the size distribution.

Conclusions. Aerosol particles going down to the nanometer range can be collected by the clCO. The nature of these particles is unknown. This supports the idea that the clCO is not limited by the size constraints of conventional air sampling systems.

General conclusions:

- The clCD provides a simple stand-alone device that can sample virus aerosols with low limits of detection.
- Aerosol particles down to the nanometer range can be sampled.
- A limited dilution method may be used to determine amplifiable particle counts with no requirement for calibration.