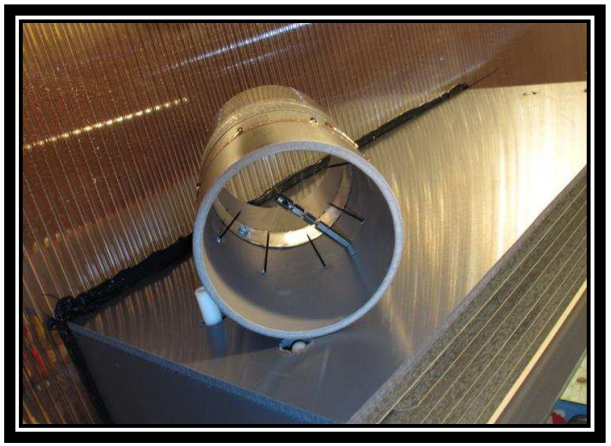
Microbiological tests of the VFA Products

Project REACT:
Resilience Enhancement Against CBRNe Terrorism
2010-2012

Tests performed at SUJCHBO – Czech Republic

Confidential

Test overview

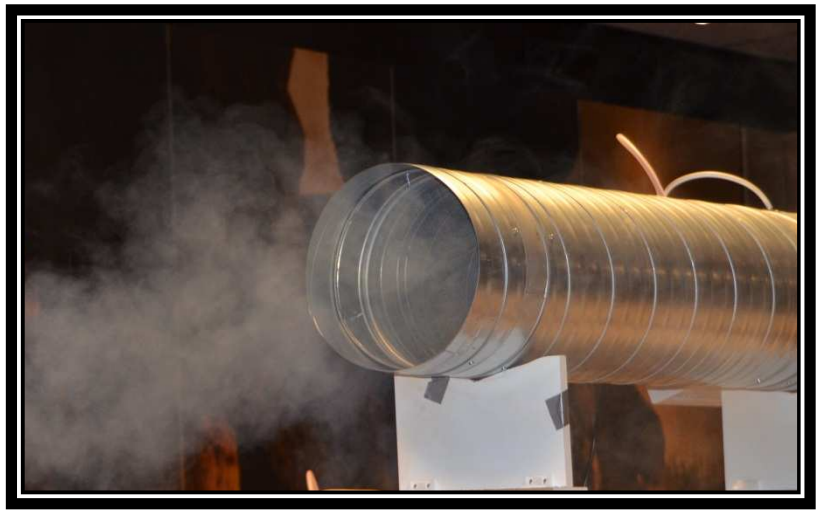


B-agent tests

- Full prototype
- Individual components (corona, ESP, coatings, HAF filter)
- Bacteria and spores
- Viruses

C-agent tests

- Full prototype
- Corona alone
- Real C-agents and simulants



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Originally desired biological tests

- pathogenic, non-pathogenic.
- **Preferred Live agents**: Bacillus Anthracis, Influenza A virus, Legionella pneumophila, Mycobaterium tuberculosis, Streptococcus Pneumoniae, MRSA, MS-2 bacteriophage virus and Noro Virus.

- **Preferred Simulants**: Bacillus globigii or thuringiensis spores, Vegetative Gram positive: Pantoeaagglomerans non sporulating bacteria, MS-2 bacteriophage virus.

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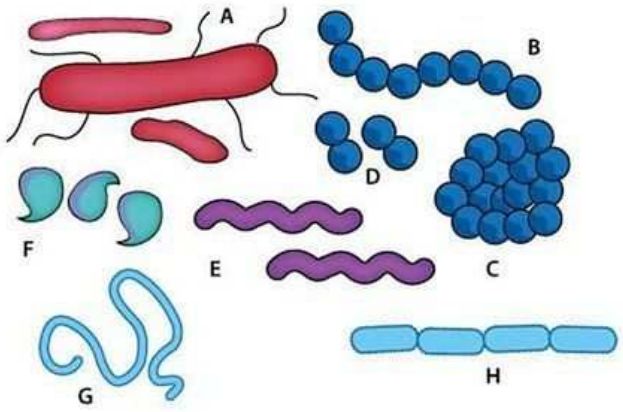
Biological test agents

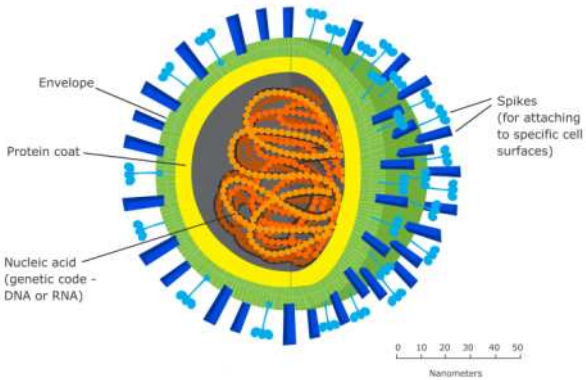
mostly relevant to nosocomial infections

- Methicillin-resistant *S. aureus* (MRSA)
- S. *pneumoniae*
- *E. faecalis*
- *E. coli*



mostly relevant as biological warfare agents

- *B. anthracis*
- *B. cereus*
- *Y. pestsi*
- *Y. enterocolitica*



- Bovine adenovirus (dsDNA)

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Chemical test agents

Simulants

- Pentyl acetate (GB/sarin substitute)
- Methyl salicylate (HD/sulfur mustard substitute)

Real agents

- Yperite (HD/sulfur mustard)
- Soman (GD).

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STAND-ALONE TEST WITH BACTERIA AND SPORES

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Stand-alone test with bacteria and spores

Experimental procedure

1. Generation of bacterial aerosol
2. Operation of the complete prototype (Corona + coated ESP)
3. Time-depend sampling of bacterial concentration, ESP wipe test
4. Background and control measurements
5. Disinfection of setup after each test
6. Incubation and reading of results

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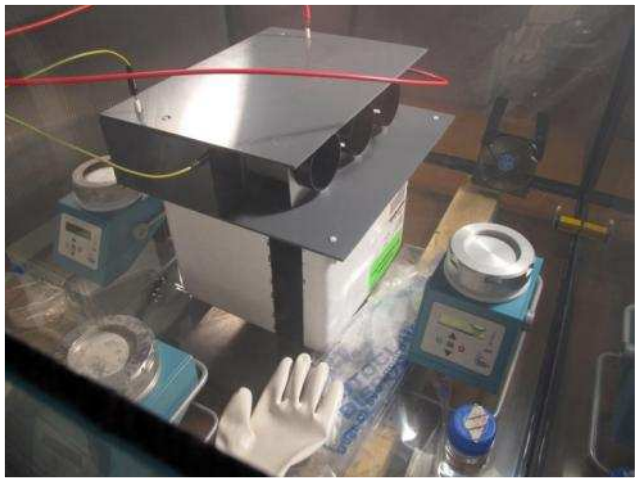
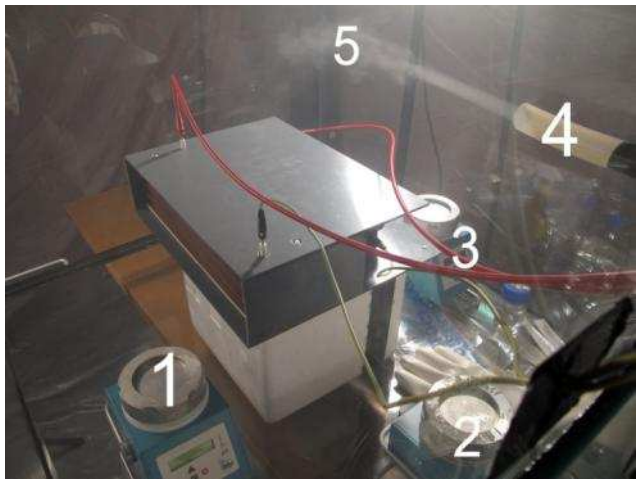
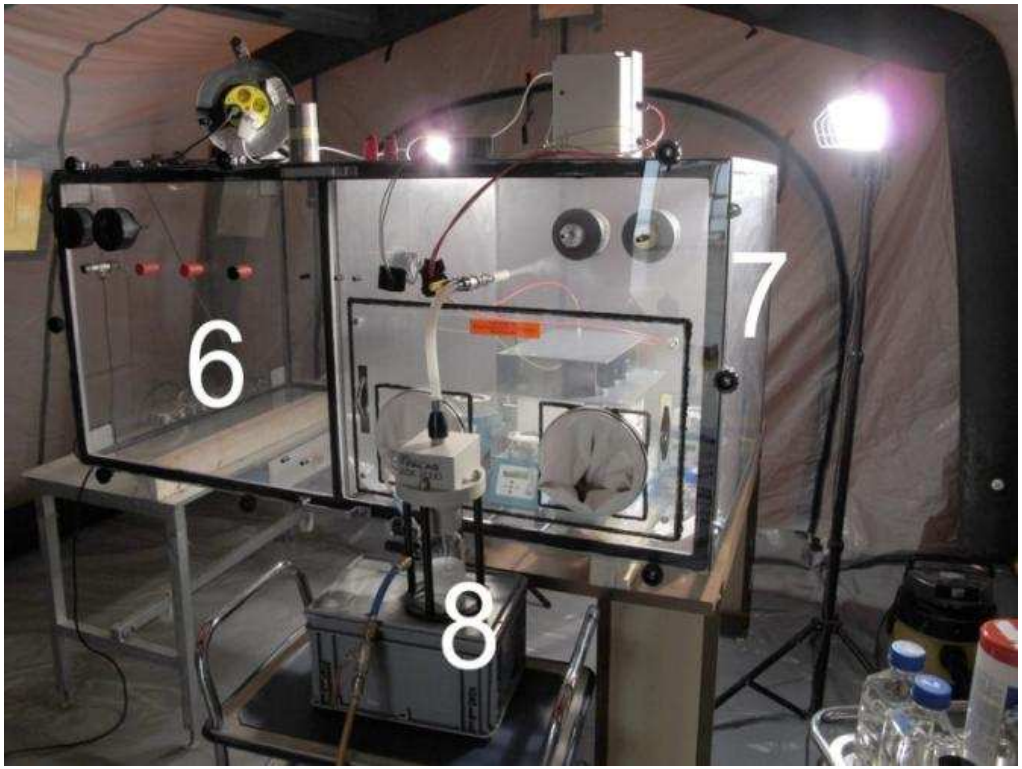
Experimental setup – complete Prototype – Stand alone







B-agent concentration: about 5E8 CFU/0.75 m3

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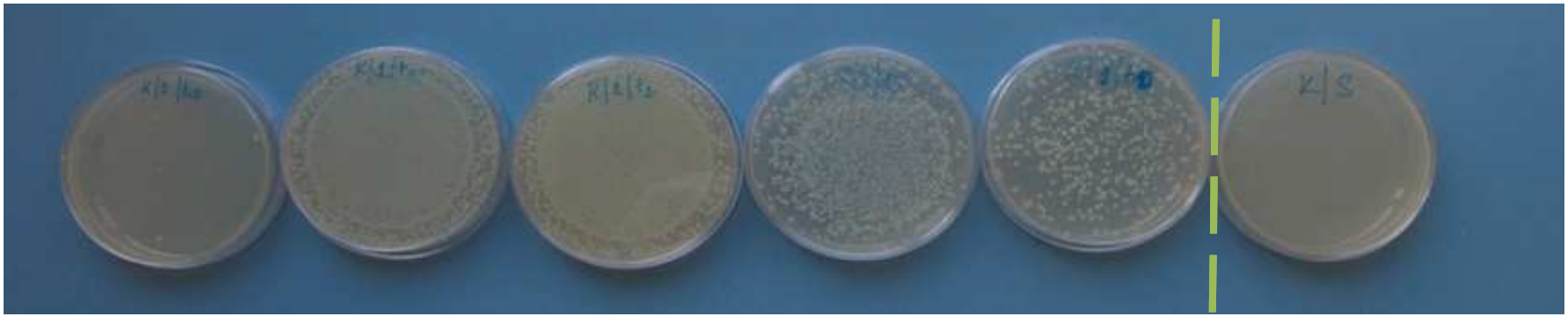
E. coli

	air sampled after				wipe test of ESP
background	0 min	2 min	5 min	10 min	30 min



prototype off

prototype on

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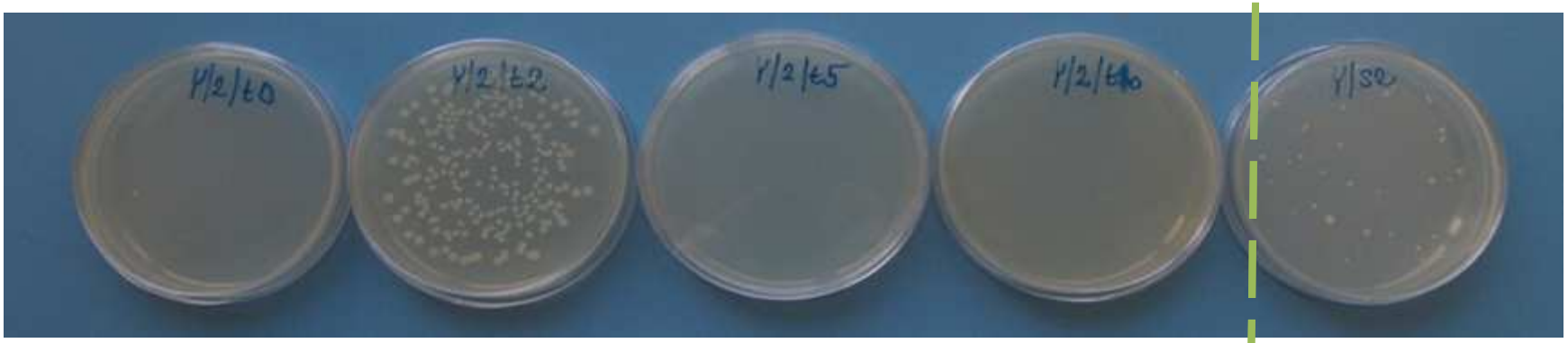
Same results for…

wipe test of ESP

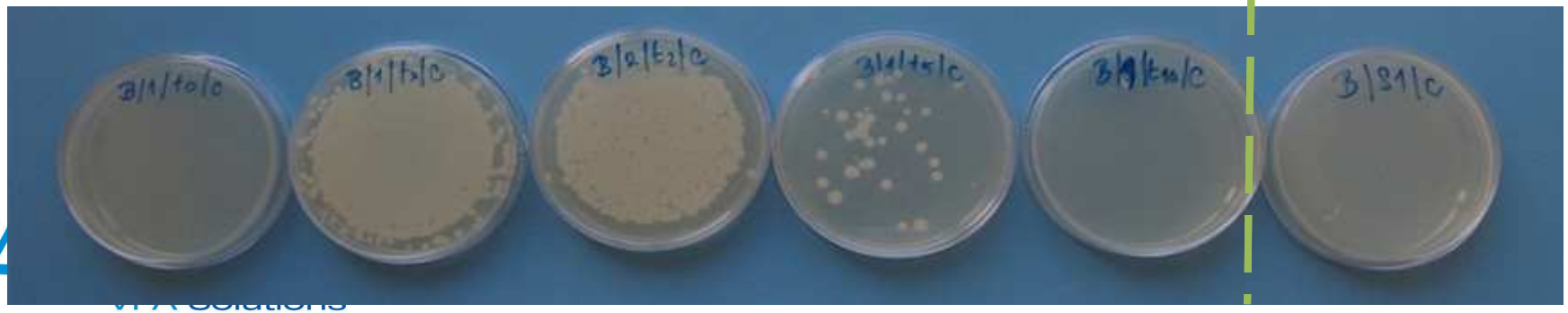
30 min

S. aureus (MRSA), G+ vegetative cells

Y. pestis, G- vegetative cells



B. Cereus, G+ spores



Summary – pathogens viability in chamber

- B. cereus spores: zero counts in less than 10 min
- Y. pestis < 5 min
- S. aureus (MRSA) < 2 min
- E. coli < 2 min
- Prototype switched off: high counts even after 10 min

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IN-DUCT TEST WITH BACTERIA AND SPORES

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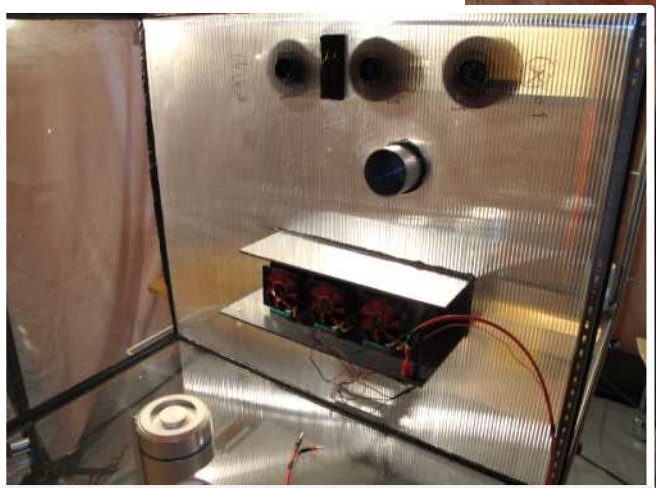
Experimental setup – Complete Protoype - INduct



B-agent concentration: about 5E8 CFU/0.75 m3
Fan speed: 0.3 m/s (0.5 m/s, 0.7 m/s)

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INduct test with bacteria and spores

Experimental procedure

1. Continuous generation of bacterial aerosol (inlet side)
2. Stabilization of aerosol
3. Adjust wind speed
4. Switch on prototype (or only corona)
5. Sampling on agar plates, ESP wipe test (outlet side)
6. Background and control measurements
7. Disinfection of setup
8. Incubation and reading of results

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Results INduct setup

	Wind speed [m/s]	*B. cereus* (spore, G+)			*E. coli* (cell, G-)			*S. aureus* (cell, G+)			*Y. enterocolitica* (cell, G-)		
	Pos. control (start)												
	BG		1		4	2	1	18	10	12	7	7	11
	0.3	149	184	196			1	3		3	5	4	7
ESP	*0.5*	208	266	212			2	1		4	53	23	47
Corona off →	*0.7*	221	312	230		1				2	14	8	17
ESP off →	*Pos. control (end)*												
Wipe tests →	*ESP*												
Corona Effect →	*BG*		2	1							3		1
ESP + corona	*0.7 m/s*	134	102	79									
ESP off →	*Pos. control (end)*										> 300	> 300	> 300
Wipe tests →	*ESP*	7	17	45									

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Testing effect of only ionisation section on Viruses

VIRUS TESTS WITH CORONA TUBES

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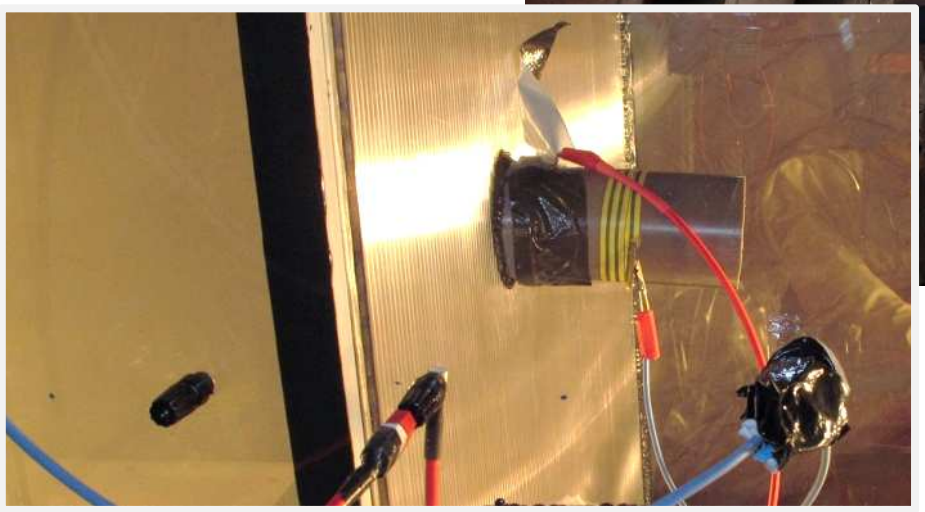
Experimental setup – Singel Pass



Virus concentration:
about 8.5E5 CFU/0.75 m3

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Virus tests with corona tubes

Experimental procedure

1. Growth of MDBK cells on Roux Bottle (RB) & Petri Dish (PD)
2. Exposure of MDBK cells (host) to Bovine adenovirus (corona *on* or *off*)
3. Time of exposure: 10 min (20 min, 30 min)
4. Incubation of cells for the following 7 days
5. Daily screening for CPE (Cyto Pathic Effect - cell destruction effect)
6. Repeated if no CPE visible after the first passage

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Results (virus tests with corona tubes)

For visual results - See page 51 in report

	Passage	Time of exposure [min] / Type of test											
		10				20				30			
		Monolayer		*Suspension*		*Monolayer*		*Suspension*		*Monolayer*		*Suspension*	
		RB	PD	RB	PD	RB	PD	RB	PD	RB	PD	RB	PD
Control													
Corona on + virus	1												
	2												
	3												
Corona off + virus	1												
	2												
	3												

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Summary

- **Corona on: No CPE even after three treatments and 21 days of incubation**
- Corona off: Strong CPE after any time of exposure even in the first passage
- Clear inhibition of virus reproduction due to corona alone.

Hypothesis:

Electron bombardment or chemical reactions alter viral surface. As a consequence host infection is hindered.

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Effect of ESP without the ionisation section (Corona Tube)

VIRUS TESTS WITH ESP

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Virus tests with ESP – Singel pass (induced air)

Experimental procedure

1. Exposure of MDBK cells (host) to Bovine adenovirus (ESP *on* or *off*)
2. Fan speed set to 0 m/s (0.3 m/s, 0.5 m/s, 0.7 m/s)
3. Incubation of cells for the following 7 days
4. Daily screening for CPE

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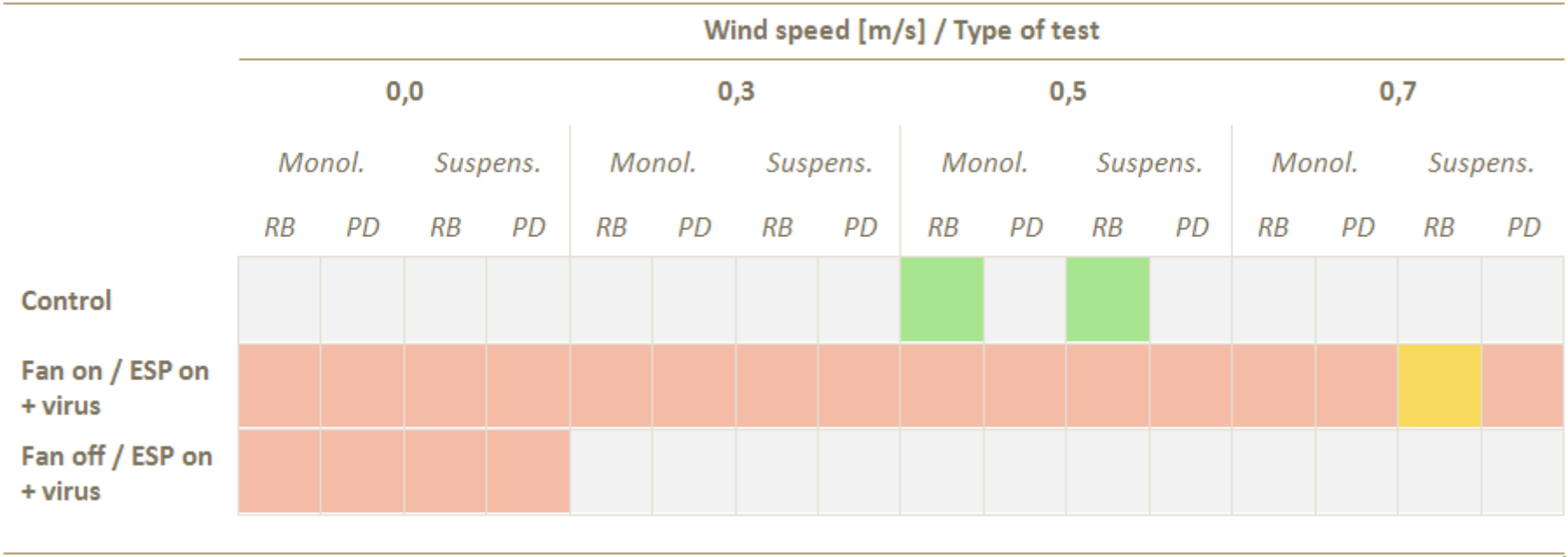
Results (virus test with ESP)



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Summary

- Strong CPE with and without ESP at any tested wind speed
- No suppresion of virus replication due to ESP

Hypothesis:

No precipitation due to a lack of charges or too low aerodynamic particle size

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Tests of complete prototype in chamber – recirculation of air through system

STAND-ALONE TEST WITH VIRUSES

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Results (SA test with viruses)

	Passage	Time of exposure [min] / Type of test					
		10		20		30	
		M	S	M	S	M	S
Control							
Corona on / ESP on + virus	1						
	2						
Corona off / ESP on + virus	1						
	2						

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Summary

- Suppression of virus replication due to the prototype
- **Switching *off* the corona results in strong CPE**
- Corona is indeed essential for virus elimination
- Effect not explained by capturing but direct elimination by corona

Hypothesis:

ESP alone not sufficient because viruses lack surface charges or show too low aerodynamic particle size

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