Investigation of artificial saliva and saline droplet size measurement for COVID-19 infection control

APMP Response Program against the COVID-19 (Project: COVID-2020-01)

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Webinar on APMP's Responses to COVID-19, 3 Nov 2022

Overview

- Introduction
- Test liquid for droplet generation
- Artificial saliva composition analysis
- Droplet size measurement methodology
- Saline & saliva droplet size measurement result
- Discussion
- Future work
- Summary

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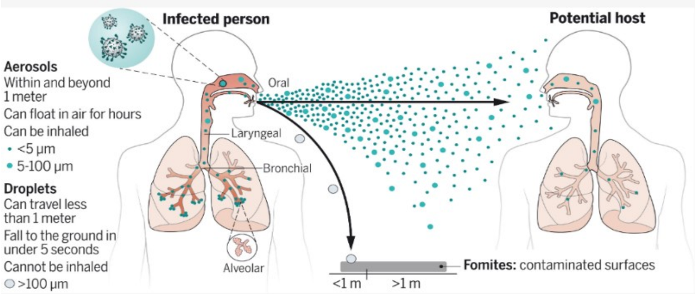
Background

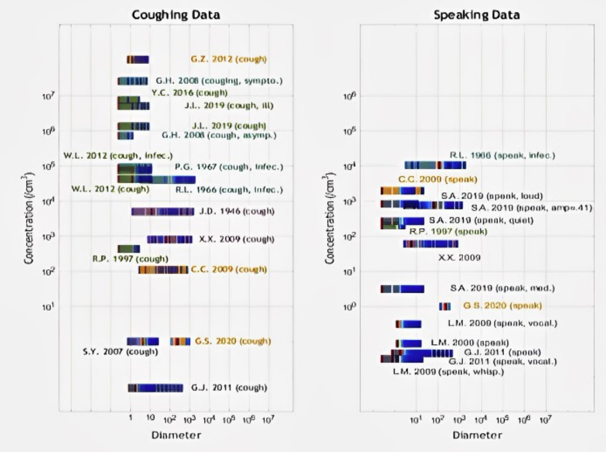
- ➢ COVID-19 virus can be spread via speech/cough droplets
  - *droplet size decides transport distance in air, suspension duration, infection risk* [1-3]

- ➢ Accurate droplet size measurement is needed

- ➢ Conflicting result in speech/cough droplet size measurement has been reported [4-8]
  - *Need for standardisation in droplet size measurement*
  - *Metrology support is needed*



Wang *et al.*, *Science* **373,** eabd9149 (2021)



Bourouiba, *US Nat. Acad. Sci., Engr., Med. - Airborne Transmission of SARS-CoV-2, A Virtual Workshop*, 2020

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Objectives

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- To choose and characterize suitable test liquid for droplet measurement
  - *Use artificial saliva droplet to simulate human speech droplet*
  - *prepare saline solutions using gravimetric method,*
  - *analyse composition of artificial saliva*
- To develop measurement methodology and evaluate droplet size measurement
  - *Droplet generation*
  - *Test chamber*
  - *Procedure, sampled by commercial particle sizers*
  - *Evaluate droplet size measurement via a inter-NMI comparison (using same droplet generator and chamber)*

Test liquid for droplet generation

- Various type of liquids have been used for droplet generation and evaporation study [13-18]
- We decided to use 6.5% & 18.1% (w/w) saline solutions and artificial saliva (with mucin, stabilized)
- saline solutions prepared using gravimetric method
  - *by dissolving high-purity NaCl (NIST SRM 919b) with Type 1 water (>18 MΩ-cm at 25° C)*
  - *purity of NIST SRM 919b: 99.835%*

Certified values for SRM919b Sodium Chloride
(from NIST certificate of analysis for SRM919b)

w_{NaCl}	99.835 %	±	0.020 %
w_{Cl^-}	60.564 %	±	0.014 %
w_{Na^+}	39.2747 %	±	0.0075 %

- Gravimetric weighing process
  - *high loading balance (Sartorius, Entris6202I-1S)*
  - *top loading balance (Sartorius, MSA323S-100-DE)*

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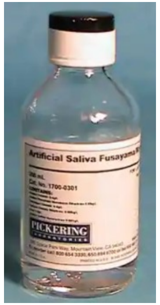
Test liquid for droplet generation

- Artificial saliva (with mucin, stabilized) was used to produce saliva droplets
  - *To simulate human speech droplet*
  - *Prepared according to ASTM E2720-16 and ASTM E2721-16 by Pickering Laboratories (Product No.: 1700-0316)*

Artificial saliva composition used for its preparation
(from ASTM E2720−16 and ASTM E2721-16)

Reagent	Amount
$MgCl_2 \cdot 7H_2O$	0.04 g
$CaCl_2 \cdot H_2O$	0.13 g
$NaHCO_3$	0.42 g
0.2 M KH_2PO_4	7.70 mL
0.2 M K_2HPO_4	12.3 mL
NH_4Cl	0.11 g
KSCN	0.19 g
$(NH_2)_2CO$	0.12 g
NaCl	0.88 g
KCl	1.04 g
Mucin	3.00 g
Distilled water	1000 mL
*p*H	7



Artificial saliva

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Mass fraction of inorganic ions in artificial saliva

- HSA employed high-accuracy methods to estimate the mass fraction of inorganic ions in artificial saliva
  - *Isotope dilution mass spectrometric (IDMS) method was employed in determination of mass fraction value (mg/kg) of total Mg, Ca, K, Cl, and $CO(NH_2)_2$* [9-11]
  - *Internal standard (ISTD) method was employed in determination of mass fraction value of total Na (mg/kg)* [11].

Analytes	Mass fraction (mg/kg)			Number of sub-samples, n
	Assigned value	Expanded Uncertainty	Coverage Factor, *k*	
Mg	20.463	0.082	2.0	3
Ca	42.74	0.43	2.6	3
K	860	35	4.3	3
Cl	1191	25	4.3	3
Na	456.5	1.2	2.3	3
$CO(NH_2)_2$	124.8	1.6	2.0	3

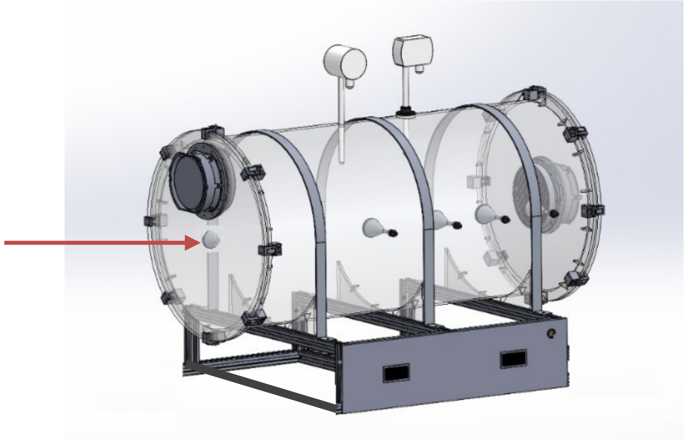
Composition analysis for artificial saliva

- NMC estimated the mass fraction of non-volatile component in artificial saliva,
  - *based on the measured mass fraction of inorganic ions*
  - *Proportional correction of the preparation mass (except for mucin) is made to match measured total ion concentration*
  - *Composition of artificial saliva was calculated*
  - *used to estimate the material density of artificial saliva droplet (after evaporation)*
- Extra amount of Mg was detected in inorganic ion measurement
  - *partially due to the preservative added (containing MgO, with its mass estimated)*
- Mass fraction of mucin cannot be measured accurately
  - *preparation mass of mucin (3.000 g) is used*

Chemical inside artificial saliva	preparation mass, g	Estimated mass based on ion measurement (except for mucin), g
$MgCl_2.7H_2O$	0.040	0.056
$CaCl_2 \cdot H_2O$	0.130	0.141
$NaHCO_3$	0.420	0.426
KH_2PO_4	0.210	0.211
K_2HPO_4	0.429	0.432
NH_4Cl	0.110	0.131
KSCN	0.190	0.192
$(NH_2)_2CO$	0.120	0.126
NaCl	0.880	0.893
KCl	1.040	1.049
Mucin	3.000	3.000
MgO	-	0.018
water	1019.362	1019.357

Droplet test chamber

- NMC designed and developed a test chamber for droplet size measurement
  - *Droplets are sprayed into the chamber*
  - *Length: ~1 m , diameter: ~0.6 m*
  - *With inlet/outlet fan and HEPA filter*
  - *Reduced particle concentration background level*
  - *Two sampling locations*

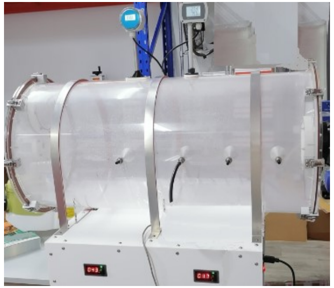


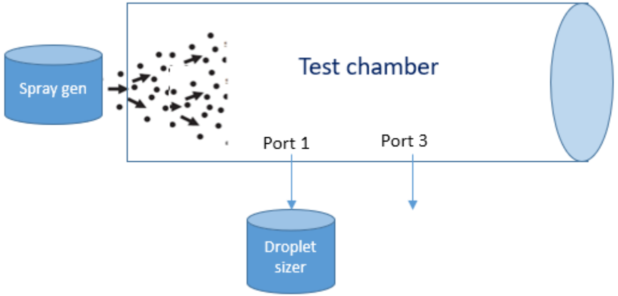
Droplet generator

- A portable ultra low volume (ULV) electric sprayer was selected as droplet generator (Longray, model: 2680A)
- produce saline and artificial saliva droplets
- good repeatability in droplet volume-size distribution:
  - *with a peak volume concentration at volume equivalent diameter (VED) around 4 μm*

Droplet size measurement methodology

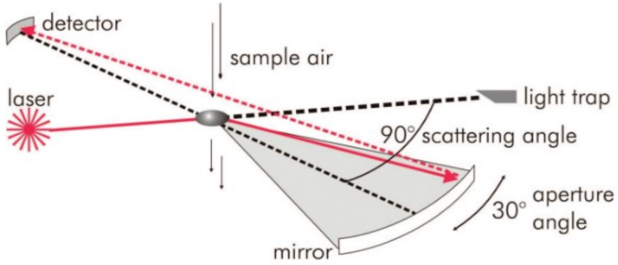
- 3 types of droplet are generated by ULV sprayer
  - *Saline 18.1% (w/w),*
  - *Saline 6.5% (w/w)*
  - *Artificial saliva*
  - *Sampling port 1 and 3 is about 40 cm and 75 cm from spray nozzle*
- Droplet VED size is measured by commercial instruments:
  - *Aerosol spectrometer (AS) is used by NMC (Grimm 11-D),*
  - *Aerodynamic particle sizer (APS) is used by CMS, NIM and KRISS (TSI 3321)* [20-21]





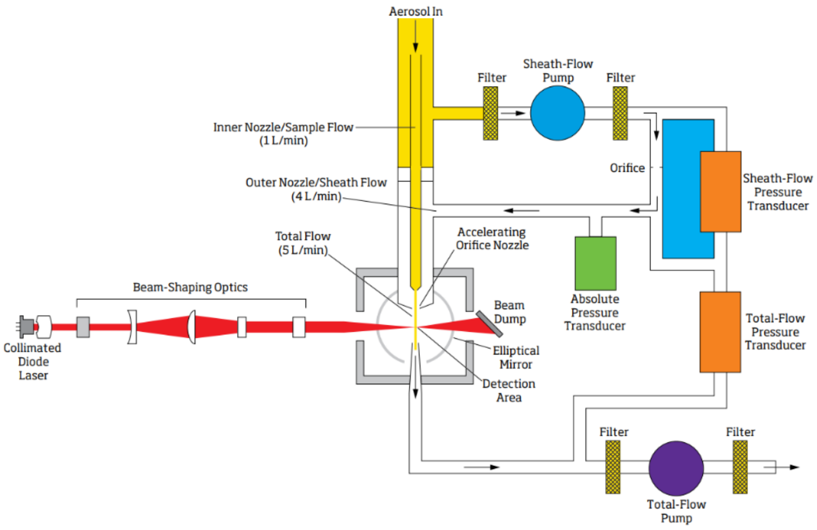
Droplet size measurement by AS & APS

- AS (Grimm 11-D)



Grimm, et al, J. Air Waste Manag. Assoc. 59 (2009)

- APS (TSI 3321)



TSI , APS model 3321 brochure

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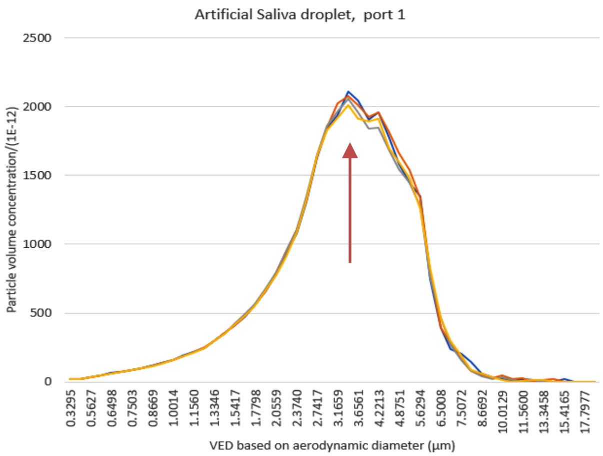
Droplet size measurement methodology

- Volume-size distribution of droplets is derived by AS or APS



- Volume equivalent diameter (VED) of each size channel *i* is given by

$$d_i = \left[\frac{d_{2,i}^4 - d_{1,i}^4}{4(d_{2,i} - d_{1,i})}\right]^{1/3} \qquad (1)$$

where ($d_{1,i}$, $d_{2,i}$) is the lower and upper bound for size channel *i* ;

total particle volume concentration for channel *i* is estimated as: $V_i = C_i\,(\pi d_i^3)/6$

- The VED (d_i) for the channel with maximum V_i is reported for evaluation of droplet size measurement

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Droplet size measurement methodology

- Optical diameter reported by AS is used to derive the VED (d_i) directly
- Aerodynamic size (d_a) from APS is converted to volume equivalent diameter d_{ve}

$$d_{ve} = \sqrt{\frac{\chi}{\rho_p}} d_a \qquad (2)$$

  - ρ_p : *droplet's material density*
  - χ : *droplet's dynamic shape factor (DSF)*
  - *DSF for NaCl and artificial saliva droplet assumed to be 1.11*

- Droplet's material density
  - *material density for saline droplet: 2.163 g/cm^3 [24]*
  - *Artificial saliva contains mucin powder (Sigma, mucin from porcine stomach, type II)*
  - *Bulk density of the mucin powder measured to be 0.681 g/cm^3 (using method A of WHO procedure [12])*
  - *Based on its composition, material density of artificial saliva droplet estimated to be: 1.196 g/cm^3*

Chemical inside artificial saliva	preparation mass, g	Estimated mass based on ion measurement (except for mucin), g
$MgCl_2.7H_2O$	0.040	0.056
$CaCl_2 \cdot H_2O$	0.130	0.141
$NaHCO_3$	0.420	0.426
KH_2PO_4	0.210	0.211
K_2HPO_4	0.429	0.432
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Mucin	3.000	3.000
MgO	-	0.018
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Evaluation of saliva & saline droplet VED measurement

- For saline droplet , measurement deviation from mean VED size was below 11.6%
- Large deviations in measured saliva droplet VED had been found:
  - *measurement deviation from mean artificial saliva droplet VED size was up to 22.7%*
  - *Higher VED deviation of artificial saliva droplet by APS (for two labs) were observed*
- VED deviation of saline droplet is much smaller than that of saliva droplet (for KRISS and CMS)

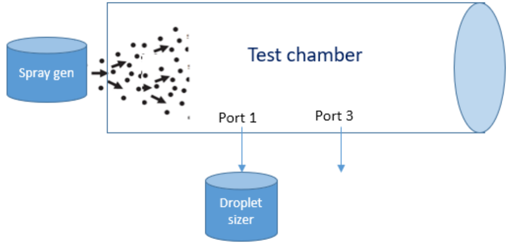
measurement at Port 1	**Mean VED size, µm**	**Relative deviation from mean VED size**			
		KRISS	CMS	NIM	NMC
Saline 18.1% (w/w)	**4.27**	-0.6%	-5.5%	2.1%	4.0%
Saline 6.5% (w/w)	**3.98**	-8.7%	-7.7%	4.8%	11.6%
Artificial saliva	**4.13**	22.7%	-20.7%	5.0%	-7.0%

measurement at Port 3	**Mean VED size, µm**	**Relative deviation from mean VED size**			
		KRISS	CMS	NIM	NMC
Saline 18.1% (w/w)	**4.44**	3.4%	-9.1%	3.8%	1.9%
Saline 6.5% (w/w)	**4.13**	-7.8%	-2.3%	5.7%	4.4%
Artificial saliva	**4.14**	19.7%	-19.0%	9.3%	-10.0%

Mean VED size (for each sampling port & droplet type) is average of measured VED by 4 labs

AS was used by NMC;

APS was used by CMS, NIM and KRISS



Discussion

- Conventional practice is to use polystyrene latex (PSL) particles as traceable reference size standard for particle sizer
- This project has shown that material density could affect APS sizing of saline or saliva droplet:
  - *Lack of traceability for saline or saliva droplet size measurement*
- PSL particle unsuitable as traceable reference size standard for saliva and saline droplet
  - *Material density of artificial saliva and saline droplet is different from that of PSL particle*
  - *Dynamic shape factor is different*
- To our knowledge, no NMI has established droplet size metrology standard for saline or artificial saliva droplet with proper traceability
  - *inter-NMI comparison of saline & saliva droplet size is only possible after traceability is established*

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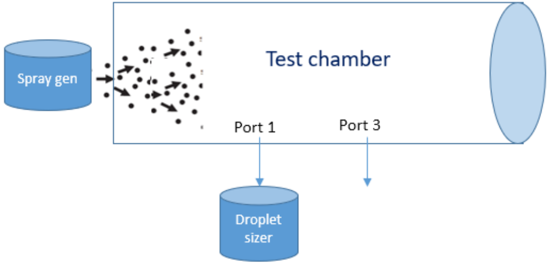
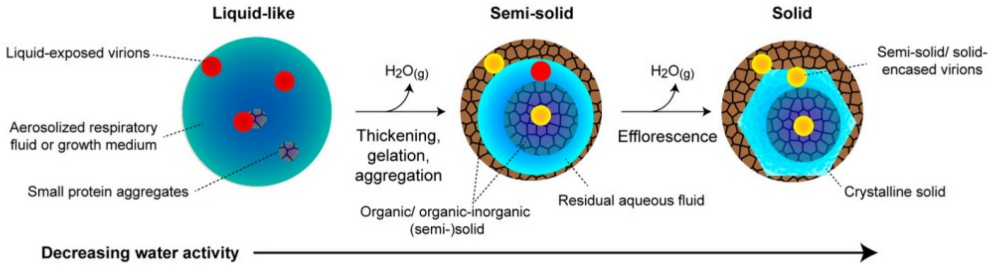
Discussion

- Possible reasons for large deviations in measured saliva droplet VED:
  - *Material density different from that of PSL*
  - *Dynamic shape factor's difference*
  - *Measurement deviation in APS due to orifice nozzle effect*
  - *Droplet generator effect on droplet size distribution*
  - *Uncertainty in droplet's density and dynamic shape factor*
  - *Evaporated saliva droplets may evolve into semi-solid phase state*





Huynh *et al.*, *PNAS*, **119** (2022)

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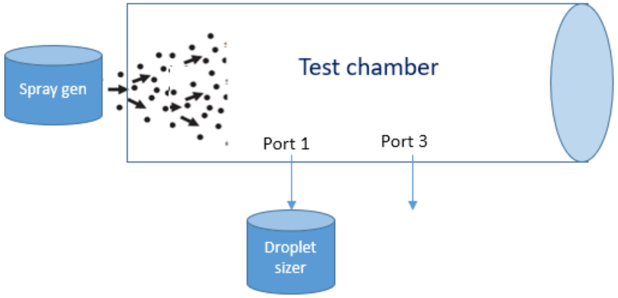
Future work

- Further R&D to address the issues in droplet size measurement
  - *develop reference droplet generator which can produce mono-dispersed saliva & saline droplets*
  - *study the dynamic shape factor of such reference droplets*
  - *study the density effect of artificial saliva and saline droplet in droplet sizing using APS*
  - *develop new reference size standard (1-20 μm) for saliva and saline droplet measurement*
  - *NMIs need to establish traceability in saliva & saline droplet measurement*

Summary

- Composition of artificial saliva is analysed to estimate density of saliva droplet
- Droplet size measurement methodology and experimental setup had been developed
  - *evaluated saline and saliva droplet size measurement for the first time among NMIs*
- AS and APS had been used to measure droplet VED size (~ 4 μm)
  - *Saline droplets' measurement deviation from mean VED size was below 11.6%*
  - *Artificial saliva droplet size measurement showed higher deviations (from mean value) up to 22.7%*
- PSL particles unsuitable as traceable reference size standard for saliva and saline droplet measurement
  - *New size reference standard is needed to establish traceability & support inter-NMI comparison*



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