Leveraging *In Vitro* Bioequivalence Tests for Locally-Acting Suspension Nasal Sprays with Three Anatomically-Correct Replicas of Human Nasal Airways Representing Intersubject Variability

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Background

- *In vitro* methods demonstrating equivalent performance are generally recommended by the U.S. Food and Drug Administration (FDA) as part of a larger approach to establish bioequivalence (BE) for generic locally-acting nasal suspension spray drug products with the reference product.

- However, due to inability of *in vitro* tests to directly measure the rate and extent of drug delivery to the site of action, *in vivo* studies are also recommended for locally-acting nasal suspension spray drug products.

- *In vivo* studies (e.g. pharmacokinetic studies) have limitations too with their sensitivity to detect formulation and device-related changes.

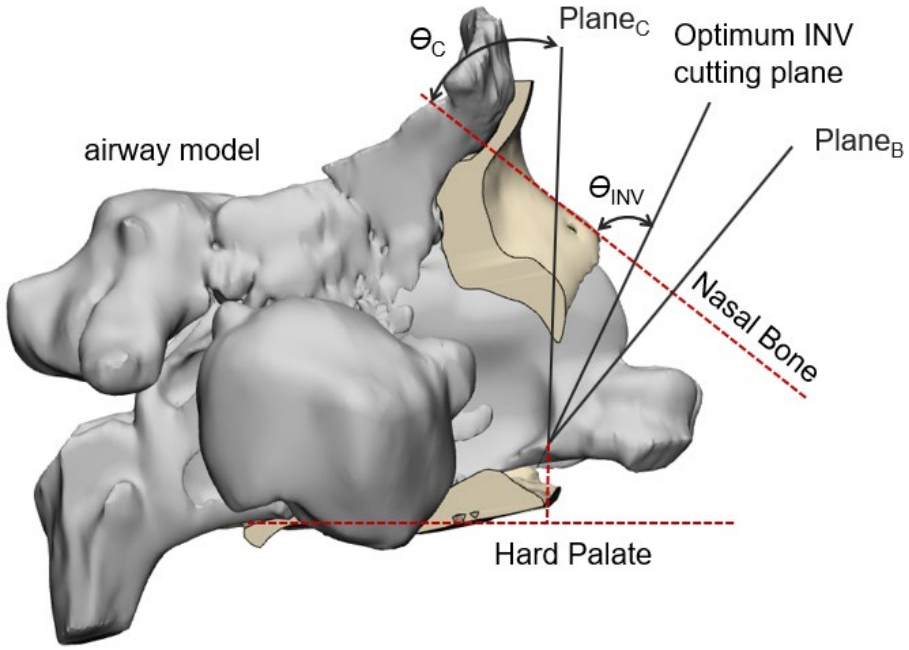
Objectives

- Our primary objective was to develop three anatomically-correct replicas of adult human nasal airways to capture the range of variability for regional deposition following administration of locally-acting nasal suspension drug products.

- The secondary objective was to evaluate any relationships between the currently recommended *in vitro* tests with regional distribution of locally-acting drugs in the three anatomically-correct replicas of adult human nasal airways.

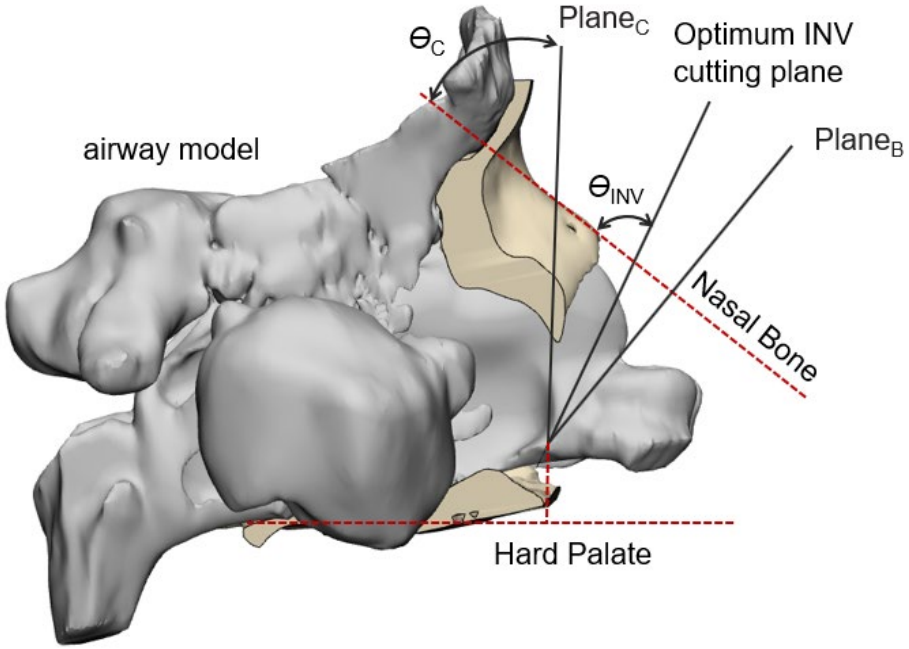
To achieve these objectives, we identified and processed sinonasal CT scans of 20 adults (50% female and 50%≥50 years, age range 21-75 years old) to develop our anatomically-correct 3D models of nasal airways that would incorporate a measure of intersubject variability.

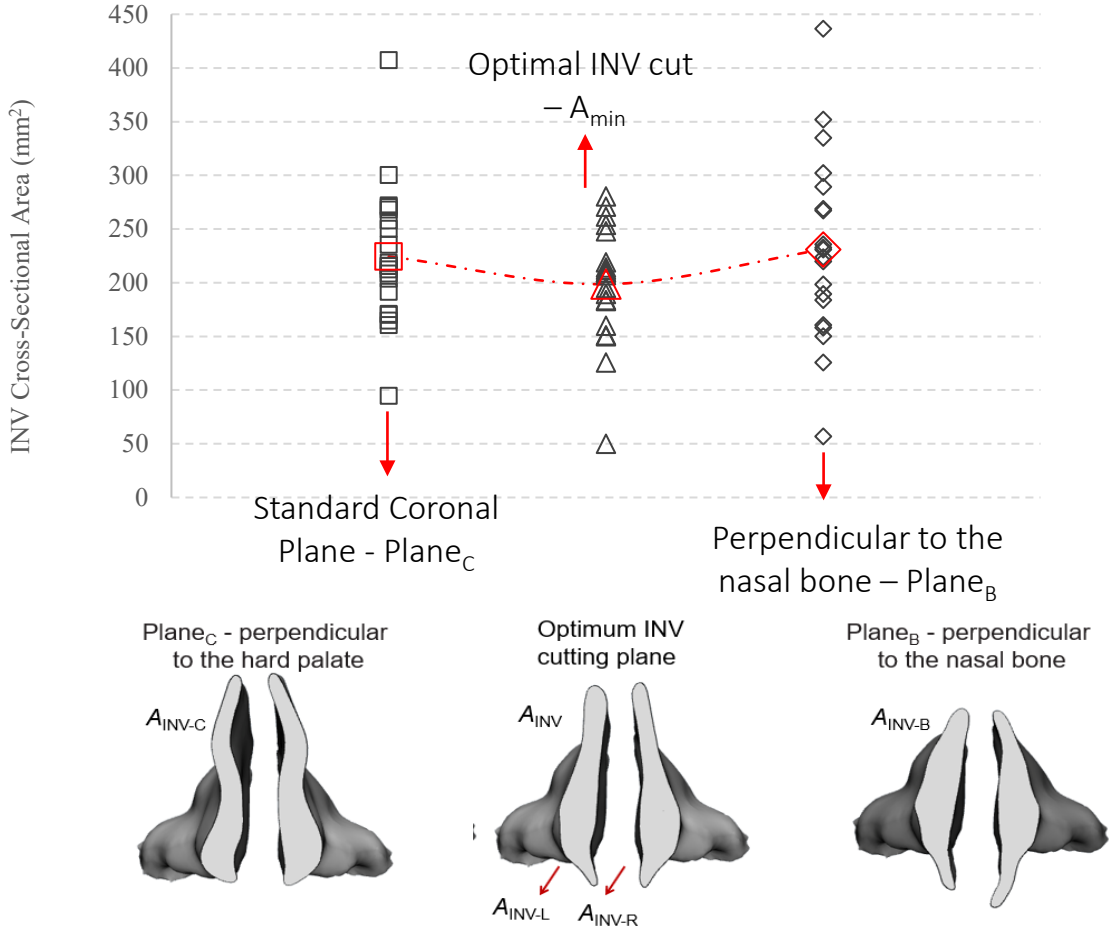
How do we define the nasal target region?
Identification of Internal Nasal Valve



Related Publication: Hosseini, S., Schuman, T. A., Walenga, R., Wilkins, J., Babiskin, A., and Golshahi, L. (2020) Use of Anatomically-Accurate 3-Dimensional Nasal Airway Models of Adult Human Subjects in a Novel Methodology to Identify and Evaluate the Internal Nasal Valve. Computers in Biology and Medicine. 123: 103896.

Identification of the Internal Nasal Valve (INV)



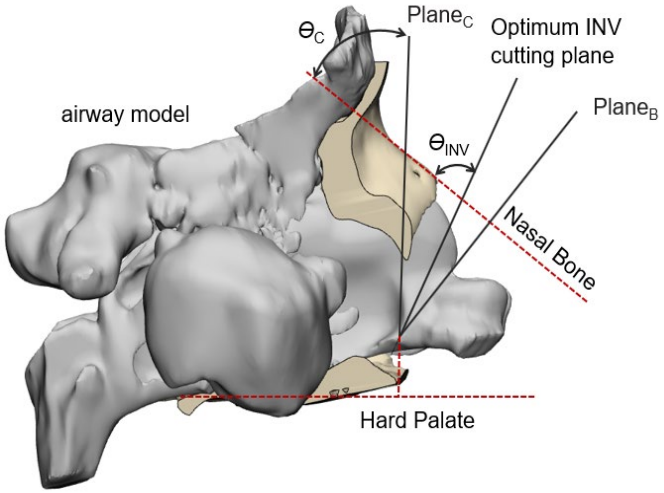


- The cut plane defines the shape and area of the INV
- The optimal cut plane results in the minimum INV cross section area while meeting anatomical definitions

Demographic and INV Characteristics of the 20 Adult Nasal Models

- The optimal cutting plane forms a mean ± standard deviation of 71 ± 7 degrees [range: 60-83°] with the nasal bone in 20 adult subjects.

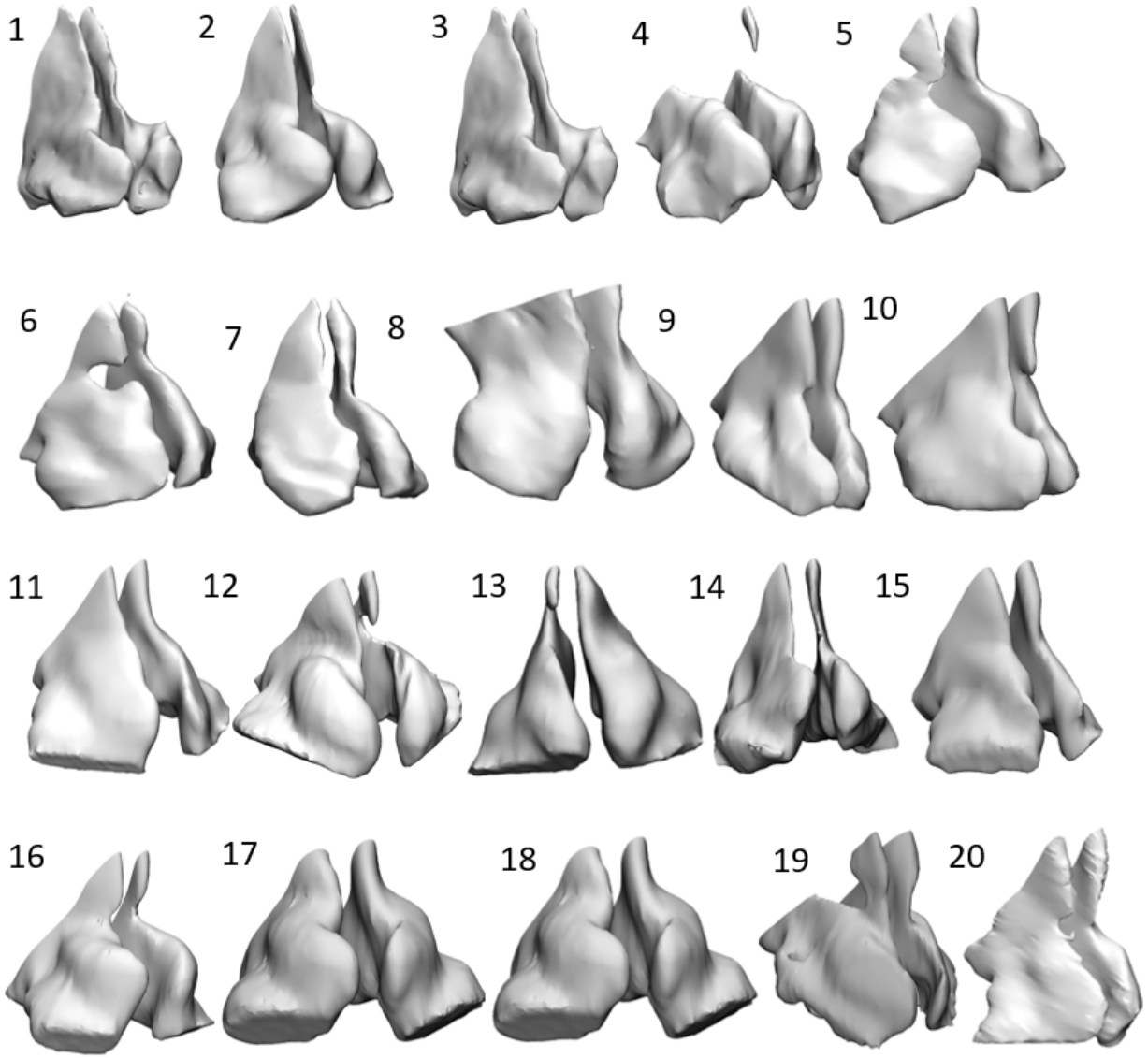
- Some of the other anatomical features of the INV were also compared with the limited *in vivo* data and showed good agreement [1].



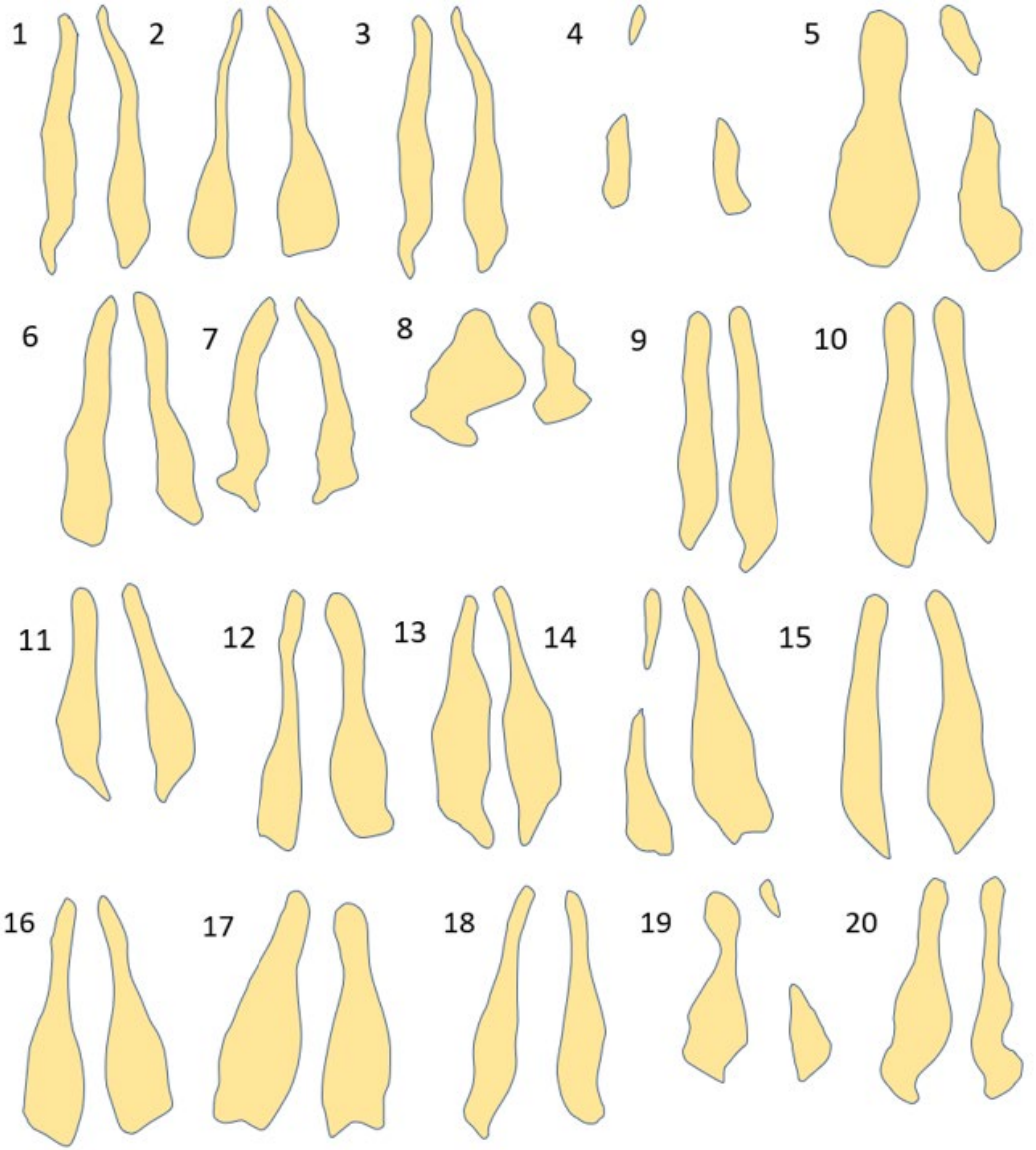
Model	Age (Years)	Gender	Ethnicity	Area of INV (mm^2)	Angle of INV (θ_{INV}, Degrees)
1	63	F	-	183	60
2	22	F	African American	144	63
3	63	F	White	152	80
4	50	F	-	50	76
5	29	M	White	254	73
6	40	M	White	209	80
7	35	M	Middle Eastern	219	68
8	50	F	African American	248	61
9	75	F	White	212	73
10	54	F	White	210	76
11	69	M	White	214	72
12	53	M	African American	205	72
13	65	M	White	280	75
14	21	M	White	165	65
15	67	F	White	207	60
16	22	F	White	191	74
17	25	F	African American	262	83
18	38	M	Middle Eastern	160	80
19	48	M	White	151	77
20	34	M	White	271	62
Average	**46**			**199**	**71**
SD	**17**			**54**	**7**

[1] Hosseini, S., Schuman, T. A., Walenga, R., Wilkins, J., Babiskin, A., and Golshahi, L. (2020) Use of Anatomically-Accurate 3-Dimensional Nasal Airway Models of Adult Human Subjects in a Novel Methodology to Identify and Evaluate the Internal Nasal Valve. Computers in Biology and Medicine. 123: 103896.

Intersubject Variability in INV, the Entrance to the Target Region, of the 20 Adult Nasal Models



Isometric View of the Anterior Region

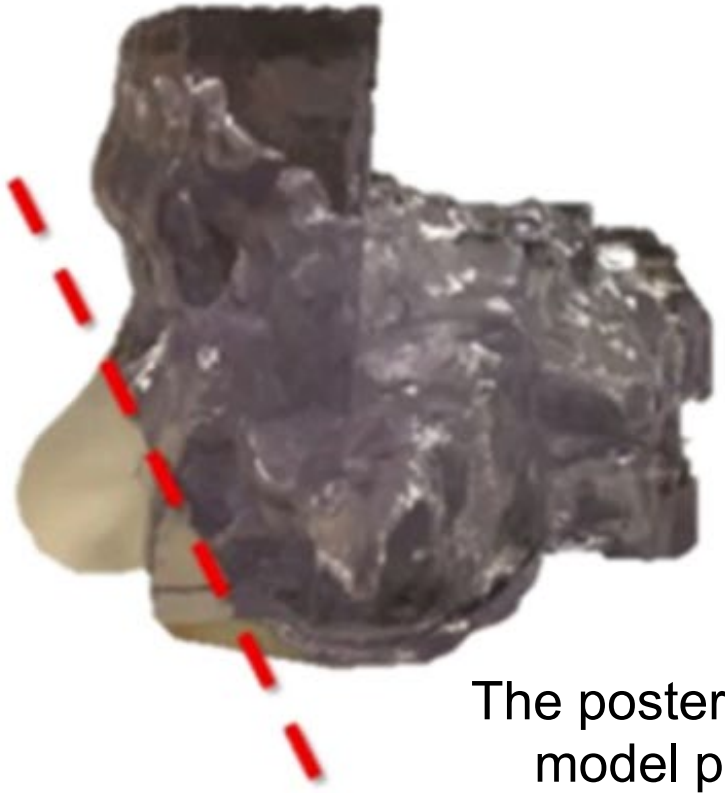


Cross-Sectional Area of the Internal Nasal Valve (INV)

How Does Intersubject Variability Affect Local Drug Delivery of Nasal Sprays?

Related Publication: Manniello, M. M., Hosseini, S., Alfaifi, A., Esmaeili, A. R., Kolanjiyil, A. V., Walenga, R., Babiskin, A., Sandell, D., Mohammadi, R., Schuman, T. A., Hindle, M., Golshahi, L. (2021) In Vitro Evaluation of Regional Nasal Drug Delivery Using Multiple Anatomical Nasal Replicas of Adult Human Subjects and Two Nasal Sprays. *International Journal of Pharmaceutics*. 593, 120103.

Rapid Prototyping Nasal Models of 20 Adults Using Stereolithography



The posterior region of the nasal model printed in clear resin

- **The posterior section: high clarity rigid plastic (Accura ClearVue)**
- **The anterior section: flexible rubbery material (TANGO PLUS 27A)**
- **40 independent nasal cavities representing the left and right sides of 20 adults**

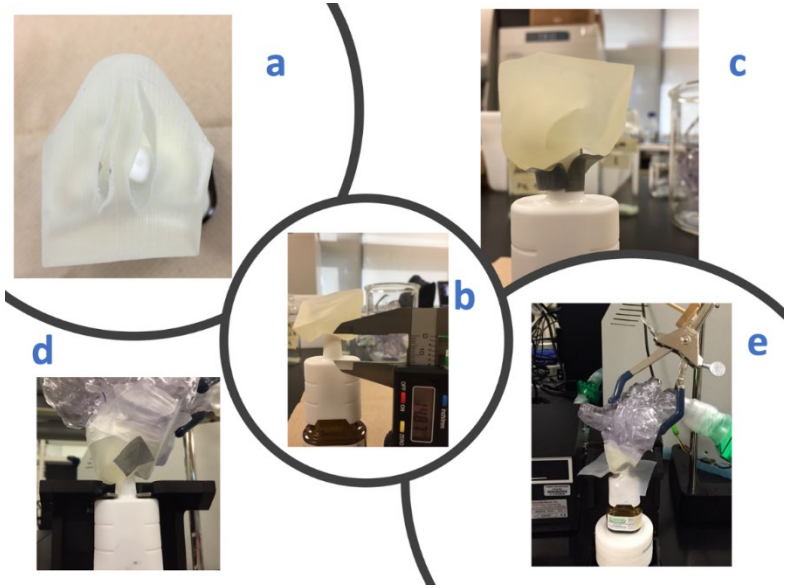
Experimental Procedure

- Test Suspension Drug Products

Flonase ®
API: Fluticasone Propionate (**FP**)
Nominal Dose: 50 μg per pray
Spray Volume: 100 μL

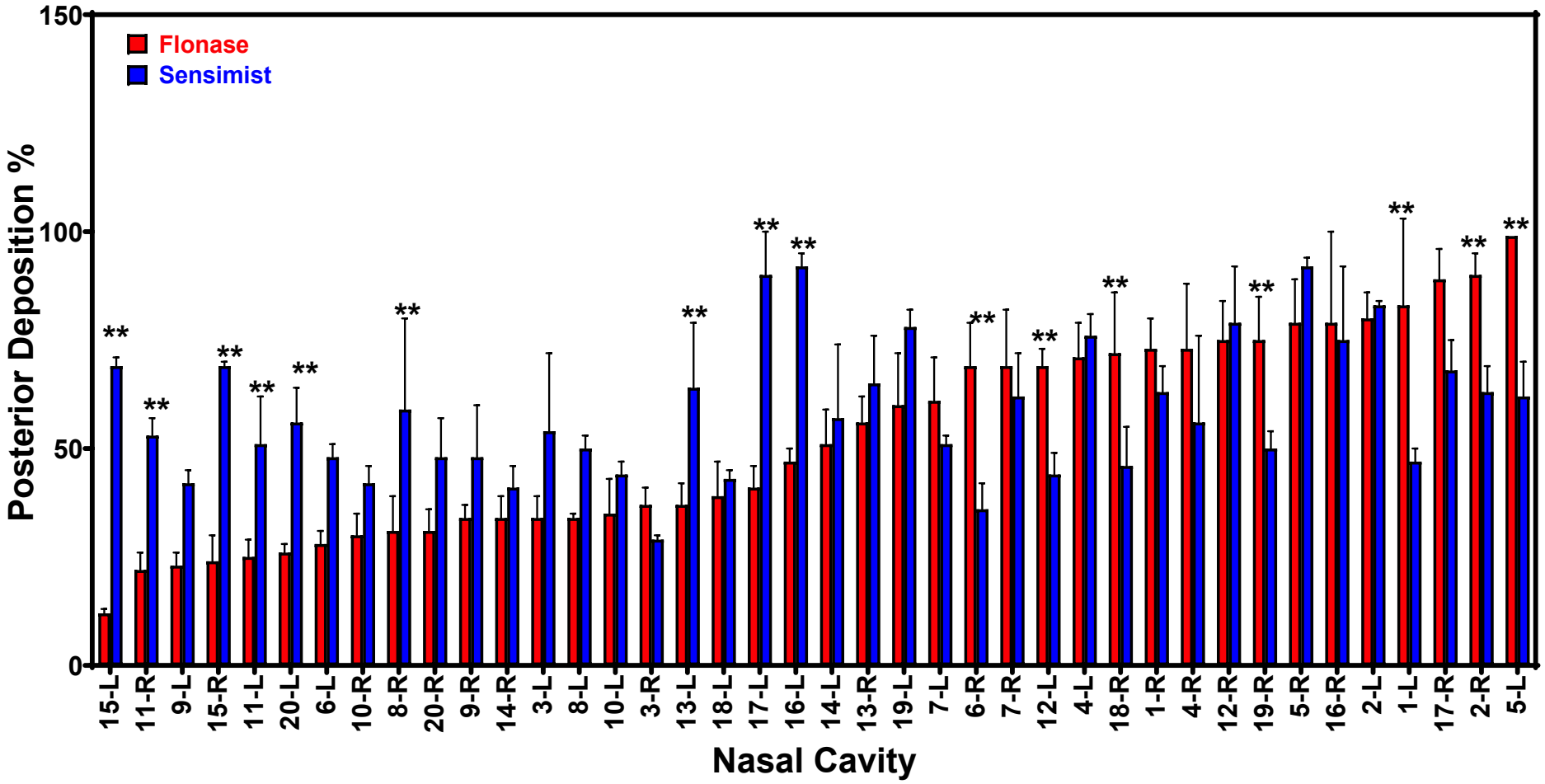
Flonase ® Sensimist ™
API: Fluticasone Furoate (**FF**)
Nominal Dose: 27.5 μg per spray
Spray Volume: 50 μL

- Model Set-Up and Administration Protocol
  - Nasal spray was inserted into the anterior region by maintaining the drug product vertically and tilting the head slightly forward.
  - It was ensured that the spray orifice was not blocked by the airway walls.
  - The insertion depth defined as total length of nozzle minus the length remaining outside the nostril was measured using a digital caliper.
  - The posterior part of the nasal replica was laid down onto the anterior part and all external borders were fitted and sealed.
  - The contralateral nostril of each replica was covered with a piece of tape.
  - The head and coronal angles were measured.
  - Automatic actuation was done at the beginning of a gentle breathing maneuver [1] using hand actuation information [2].



[1]Guo et al. *Pharm Res* 2005, 22: 1871-8.
[2] Doughty et al. *Drug Dev Ind Pharm* 2011, 37: 359-66.

Intersubject Variability of Local Drug Delivery to Target Region for Nasal Sprays



Posterior region (circled)

Range of FP: 12-99% of dose in target regions **Range of FF:** 29-92% of dose in target regions

** = significant difference between the 2 groups (observed in 16 of 40 nasal cavities).

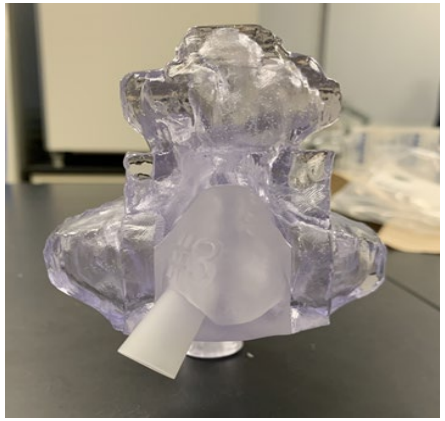
Identification of Three Nasal Airway Models Representing Low, Mean, and High Posterior Deposition (PD) for Suspension Nasal Sprays

Selection of 3 Nasal Models Representing Low, Mean, and High Posterior Deposition (PD)

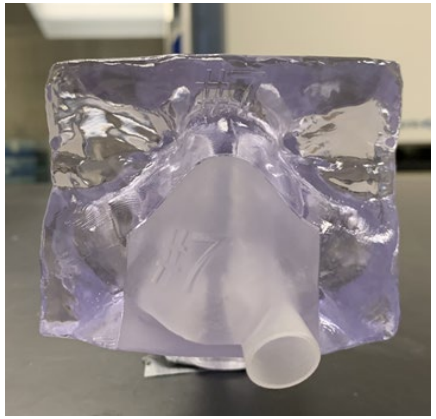
Selection Criteria:

1) The few nasal cavities showing significantly different posterior deposition (PD) with the two nasal sprays were excluded.
2) The PD in the models representing minimum (L-Model for low PD), mean (M-Model), and maximum (H-Model for high PD) should be significantly different to represent three distinct levels of PD.
3) The M-Model should show a PD not significantly different from the mean PD, obtained for each of the two drug products.

Low PD (L-Model): Within two standard deviations below the mean (mean-2SD)
Mean PD (M-Model): Mean or no significant difference with mean
High PD (H-Model): Within two standard deviations above the mean (mean+2SD)



Low PD (L-Model)



Mean PD (M-Model)

High PD (H-Model)

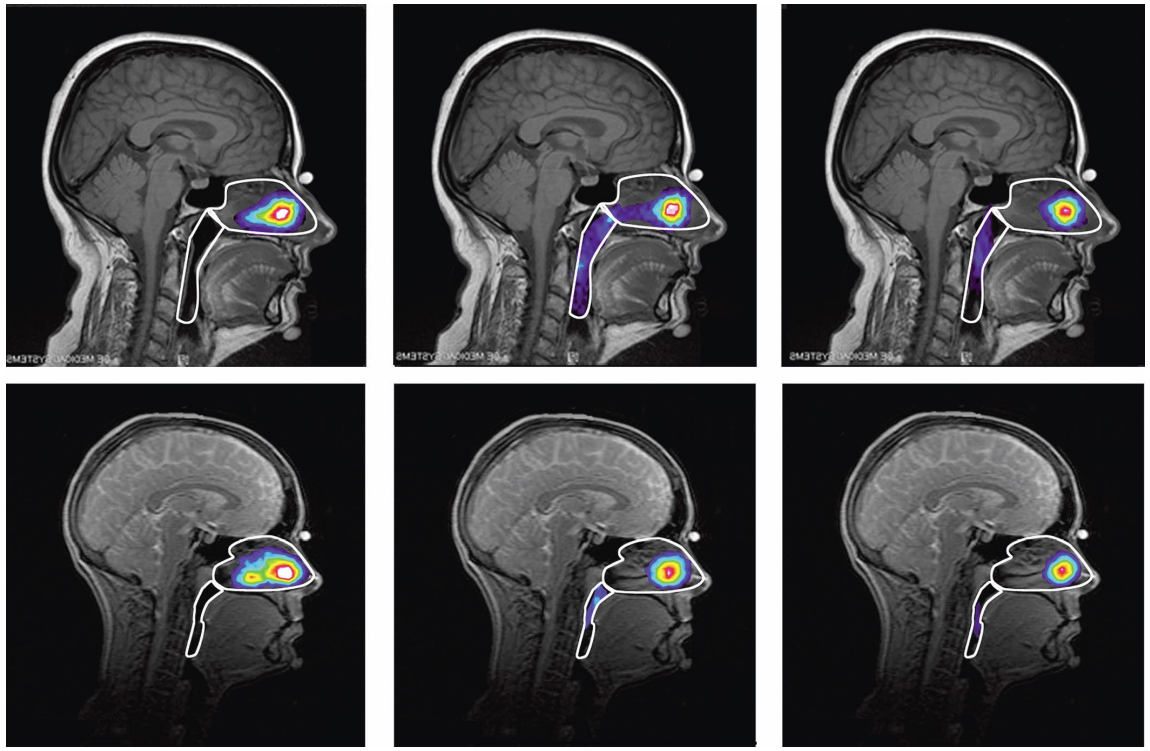
Characteristics of 3 Nasal Models Representing Low, Mean, and High Posterior Deposition (PD)

Posterior Deposition	Replica/ Nostril	Age (Years)	Gender	Mean ± SD PD (% Recovered Dose FP)	Mean ± SD PD (% Recovered Dose FF)	Mean ± SD PD (% Recovered Dose MF)
Low (L)	3 – Right	63	F	36.5±4.0	28.8±1.0	24.5± 3.9
Mean (M)	7 – Left	35	M	60.5±10.0	51.4±2.0	46.7±7.6
High (H)	2 – Left	22	F	80.3±6.0	83.5±1.0	72.7±2.4

*MF - Nasonex ®API: Mometasone Furoate Monohydrate (**MF**); Nominal Dose: 50 μg per spray; Volume: 100 μl per spray

Comparison of *In Vitro* Deposition in L, M, H, Models with *In Vivo* Deposition

- Same subject *in vitro-in vivo* comparison was not possible for our three selected models.
- An *in vivo* study [1] performed using Nasonex (mometasone furoate, MF) was replicated using the three *in vitro* models.



[1] Shah SA, Berger RL, McDermott J, Gupta P, Monteith D, Connor A, et al. Regional deposition of mometasone furoate nasal spray suspension in humans. Allergy Asthma Proc. 2015;36:48–57.

Comparison of *In Vitro* Deposition in L, M, H, Models with *In Vivo* Deposition

- ***In vivo* Deposition [1]**
  - 12 Caucasian adults (10 females and 2 males) aged 23-64 years old
  - Mean ± SD= 60±9%; Range: 53% - 67%

- ***In vitro* Deposition in L, M, H Models:**
  - 20 multi race adults (50% female and 50%≥50 years, age range 21-75 years old)
  - 47±8% in M-model; Range: 24% - 73%

Potential variation were due to:

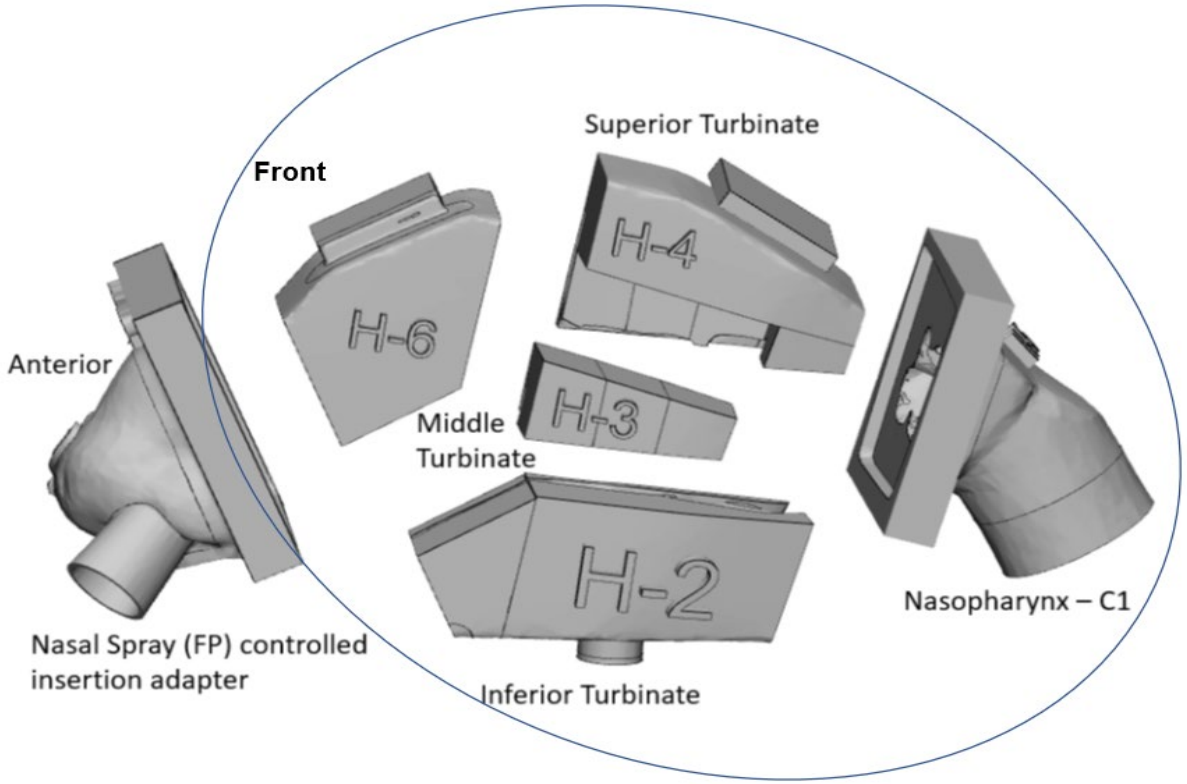
- **Differences in defining target regions**
- Differences in administration
- Cumulative deposition for *in vivo* (four shots in both sides of septum) vs *in vitro* (two shots per side, independent from the other side)

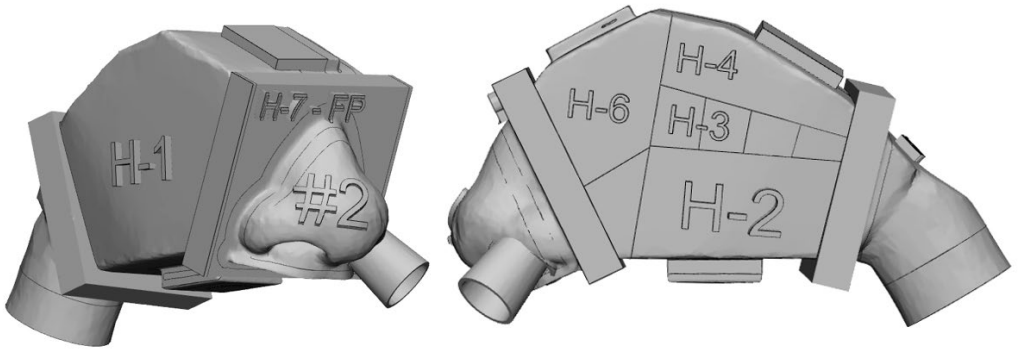
[1] Shah SA, Berger RL, McDermott J, Gupta P, Monteith D, Connor A, et al. Regional deposition of mometasone furoate nasal spray suspension in humans. Allergy Asthma Proc. 2015;36:48–57.

Can the L, M, H Models be Used to Provide Regional Drug Distribution in the Target Region?

Development of Regionally Sectioned L, M, H Models

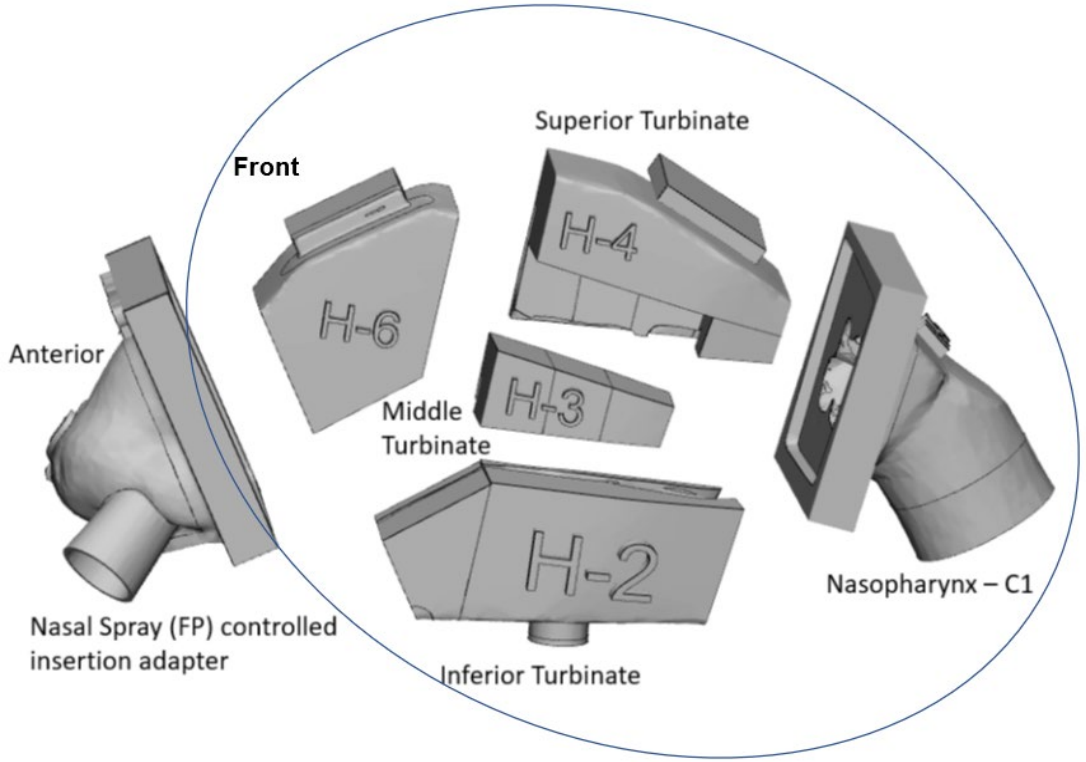
- **Posterior region of the models were segmented into 5 pieces:**
  - Front
  - Superior turbinate
  - Middle turbinate
  - Inferior turbinate
  - Nasopharynx





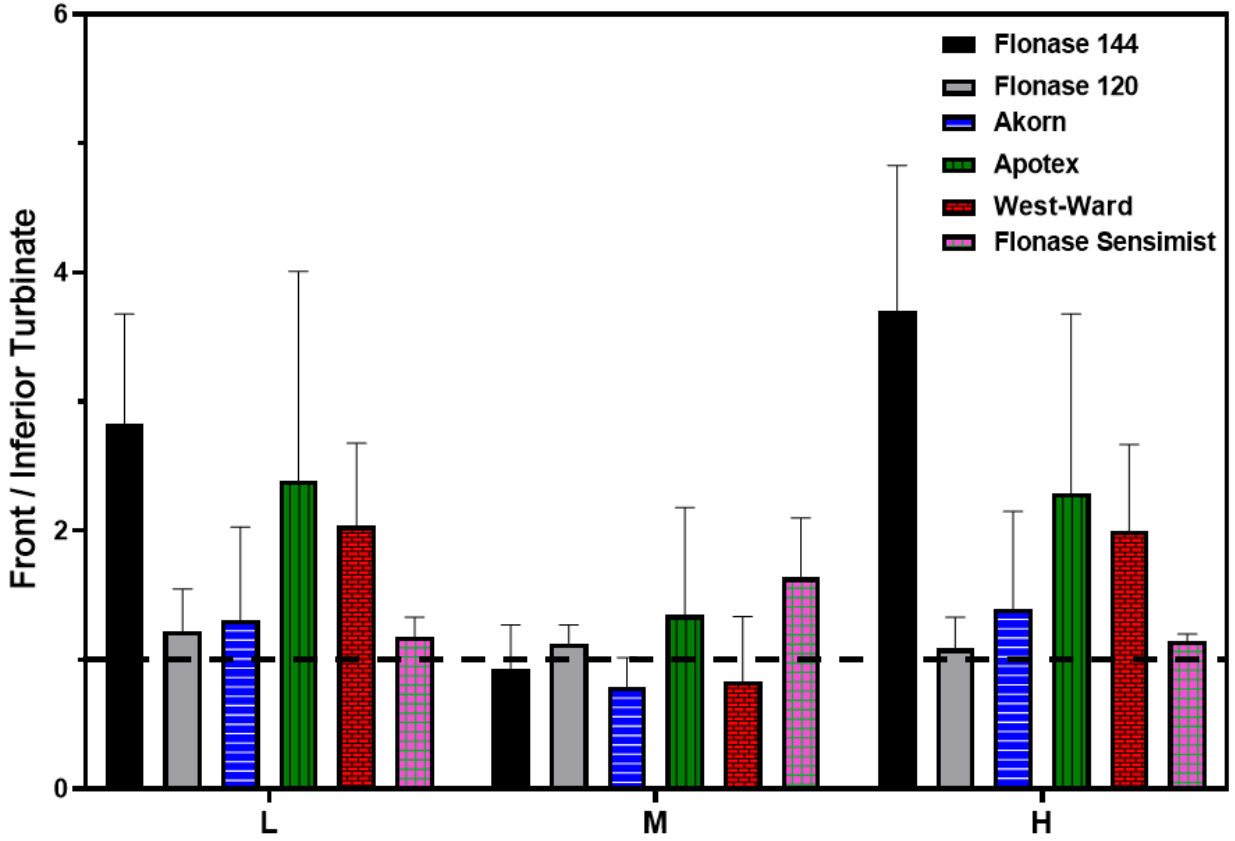
Regional Deposition of FP Nasal Suspension in L, M, H Models

Posterior Section	Mean ± SD PD (% Recovered Dose FP) in H-Model	Mean ± SD PD (% Recovered Dose FP) in M-Model	Mean ± SD PD (% Recovered Dose FP) in L-Model
Anterior	23.2±4.2	52.5±0.8	64.1±0.9
Front	37.9±4.5	22.4±1.1	15.8±1.2
Inferior	32.4±6.1	20.0±2.1	17.2±1.0
Middle	3.0±2.4	3.0±1.4	1.2±0.5
Superior	1.6±1.0	2.3±1.1	0.0±0.0
Nasopharynx	1.8±0.2	0.0±0.0	0.7±0.1



Front & inferior turbinate regions received >90% of PD.

Comparison of Front to Inferior Turbinate Ratios in RLD (Flonase 120 Sprays) and Generic FP Nasal Products



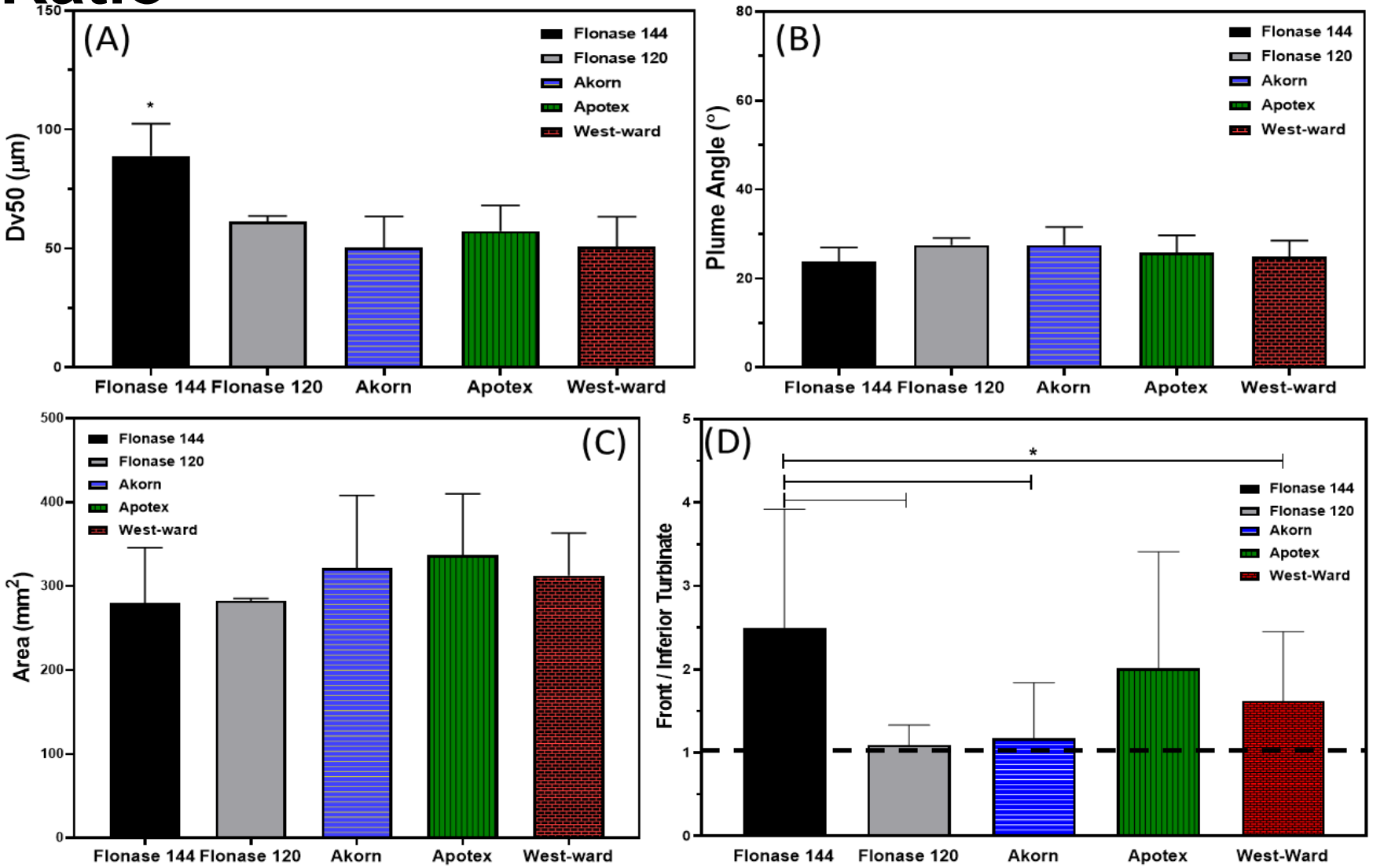
Comparison of three generic FP products (Akorn, Apotex, and West-Ward) with the RLD Flonase (containing 120 sprays), the over the counter drug product Flonase Allergy Relief (containing 144 sprays), and Flonase Sensimist (FF) as the active ingredient shows:

(1) Front to Inferior Turbinate ratio (F/IT) can be a potential metric as part of a large approach to establish BE for generic nasal suspension drug products

(2) Ideally, F/IT should be maintained at ≤1

Is there a Relationship Between Current *In Vitro* Tests and Local Nasal Deposition in the L, M, H Models?

Characterization of RLD and Generic FP Nasal Products Using Current *In Vitro* Tests and Front / Inferior Deposition Ratio



- No strong relationships between current *in vitro* test method metrics and regional nasal deposition in models have been found so far.
- However, the pooled regional deposition in the L, M, and H Models showed that an increase in Dv50 may indicate higher deposition in the front region leading to a higher ratio of front to inferior turbinate region.

Conclusions

- We have identified three realistic nasal airway geometries that represent the low, mean and high posterior nasal cavity deposition in adults measured for two nasal spray products.

- The models have been segmented to allow for regional differences in deposition to be evaluated. However, no strong relationships between current *in vitro* test method metrics and regional nasal deposition in models have been found so far.

Acknowledgements

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Views expressed in this talk do not necessarily reflect the official policies of the U.S. Food and Drug Administration, nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

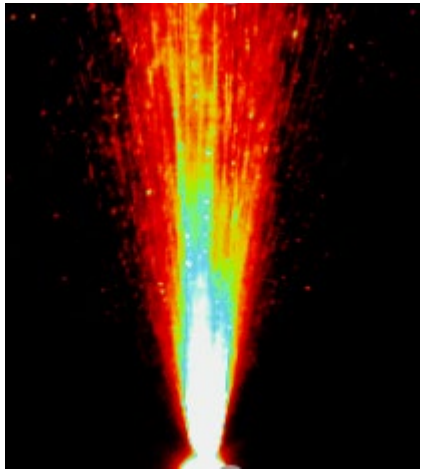
* All last names are listed in alphabetical order and the order does not necessarily reflect the order of contributions.

Table 1. The administration parameters, including the insertion length and the head and coronal angles, used for Flonase and Flonase Sensimist nasal sprays.

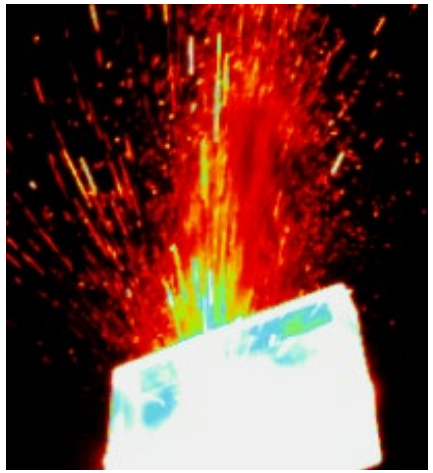
Model #	Insertion Length (mm)		Head Angle (°)				Coronal Angle (°)			
	Flonase		Flonase		Flonase Sensimist		Flonase		Flonase Sensimist	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
1	18	14	45	51	54	47	36	30	45	29
2	12	12	45	53	50	46	20	31	32	35
3	9	10	42	56	55	50	38	29	50	22
4	12	13	59	52	54	61	39	26	34	7
5	16	14	58	57	58	59	31	14	36	14
6	15	11	48	43	46	58	18	36	25	37
7	16	16	45	57	48	59	25	31	23	29
8	14	11	56	51	53	60	37	33	41	41
9	11	11	56	52	55	62	39	32	41	42
10	12	12	44	59	39	63	33	46	35	32
11	15	16	39	51	40	59	35	40	43	37
12	18	15	50	55	44	57	44	55	38	56
13	14	15	28	33	33	45	26	23	46	31
14	11	9	48	48	47	57	46	47	33	25
15	12	12	56	54	52	59	46	44	30	28
16	11	9	47	55	51	57	29	49	31	32
17	14	13	59	50	49	54	23	38	46	42
18	17	16	47	47	52	56	15	43	30	36
19	8	9	49	45	48	54	38	46	33	37
20	14	14	50	50	43	53	32	42	38	41
Mean ±SD	13±3	13±2	49±8	51±6	48±6	56±5	33±9	37±10	37±7	33±11
p-value [a]	0.43		0.27		<0.001		0.18		0.18	
Mean ±SD [b]	13±3		50±7		52±7		35±10		35±9	
Range [b]	[8-18]		[28-59]		[33-63]		[14-55]		[7-56]	

Plume Characterization at INV

- Some of the test metrics (width, area, and ovality) were re-examined at a distance that is relevant to the nasal models, specifically, at the distance between the nasal tip and the internal nasal valve (INV) in the H model.
- Plume was also re-examined as goes through the anterior regions of nasal models.



Plume Geometry (PG) Westward



High Model PG Westward

Low Model PG Westward

Size distribution of West Ward going through the anterior of the H model

		Without (μm)	With (μm)
Average	Dv10	22.09	23.79
SD		1.48	1.24
Average	Dv50	68.80	63.30
SD		4.18	1.93
Average	Dv90	112.62	96.25
SD		3.42	0.61

Spray Pattern at INV

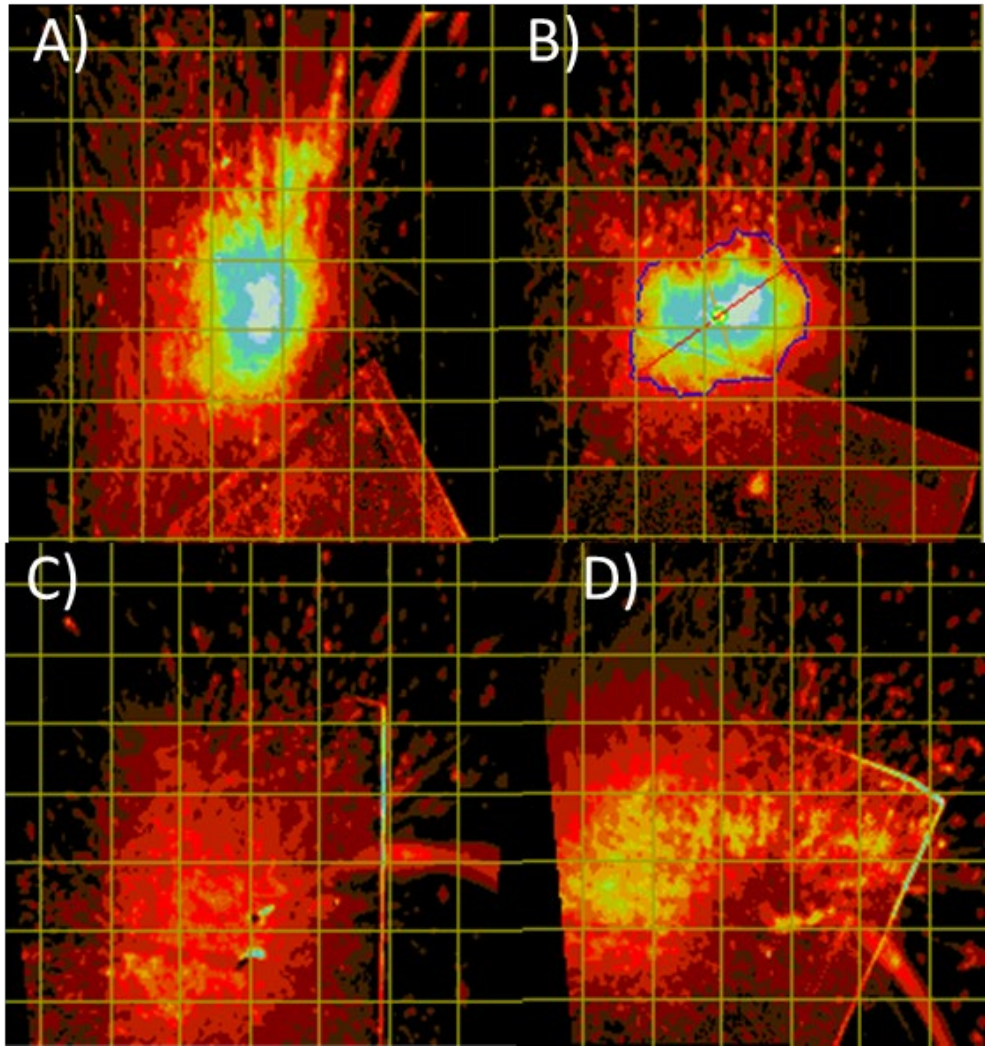
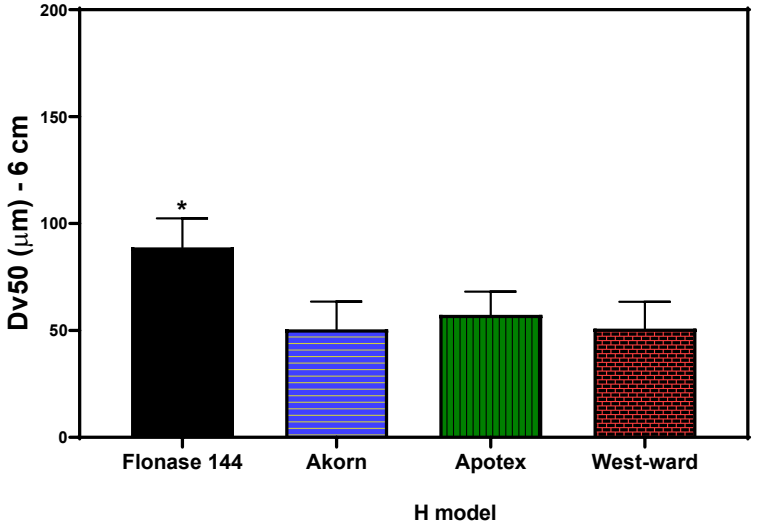
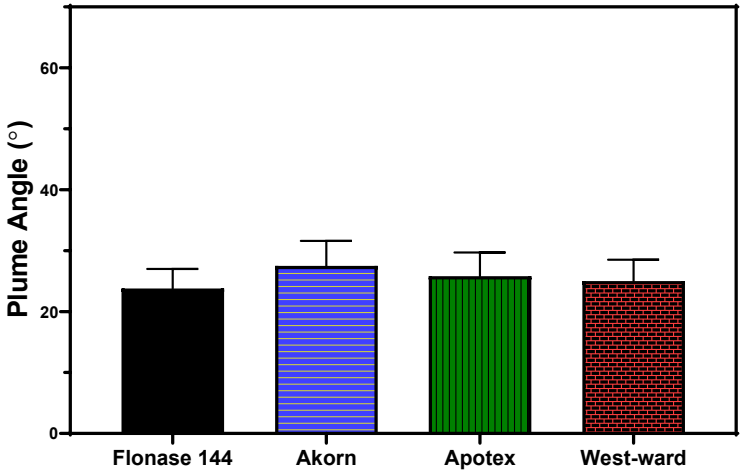


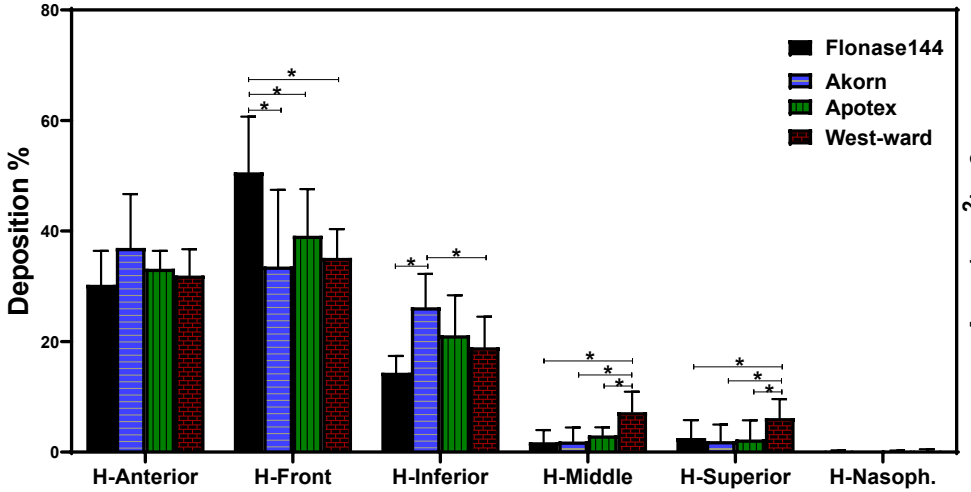
Figure 4. Spray pattern at 60 mm from the tip of West-ward. A and B show two replicates with the H model attached while C) and D) show the pattern with the L model attached.

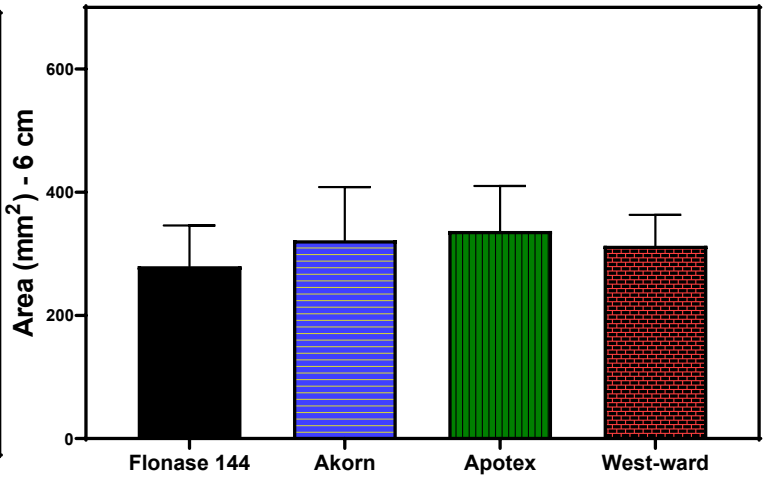
BE Test Metrics Vs. Regional Deposition: H Model

- H Model➔ regional deposition% differences in front and inferior turbinate regions with the highest regional deposition.
- H model Front➔ F144 is significantly different than three generics FPs
  - F144 ➔ significantly higher Dv50
- Inferior turbinate shows possible trend due to Dv50. F144 has the highest and Akorn has the lowest Dv50, but also West-ward vs Akorn different without relevance to Dv50.
- Middle & superior ➔ Westward vs others, but total =<7%



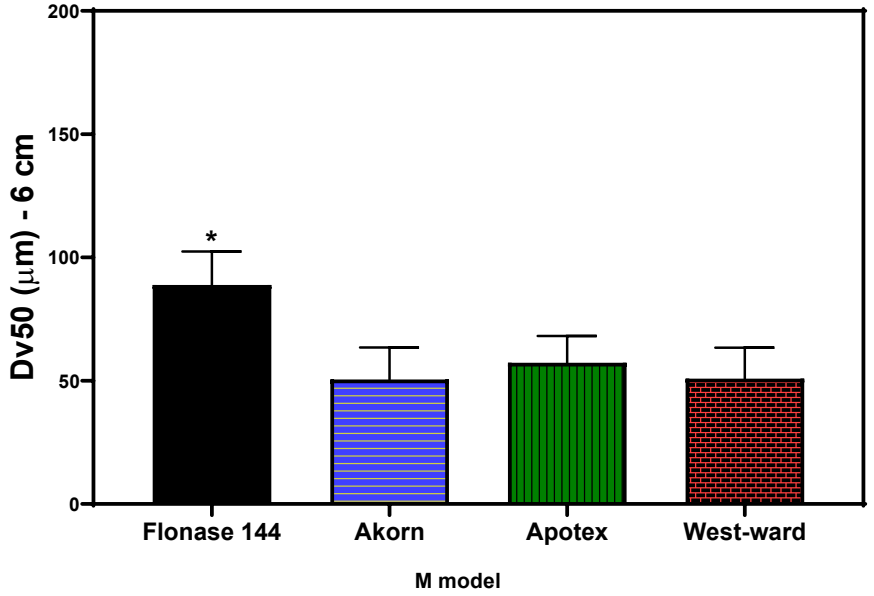


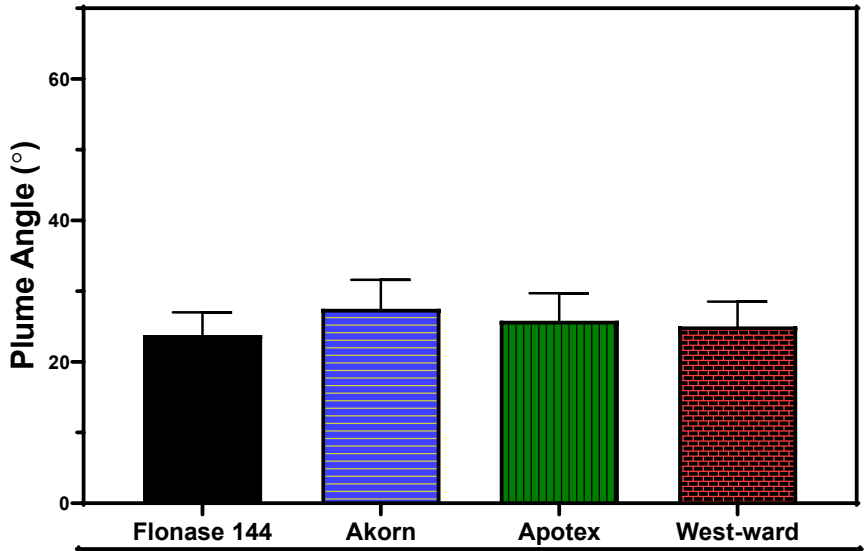


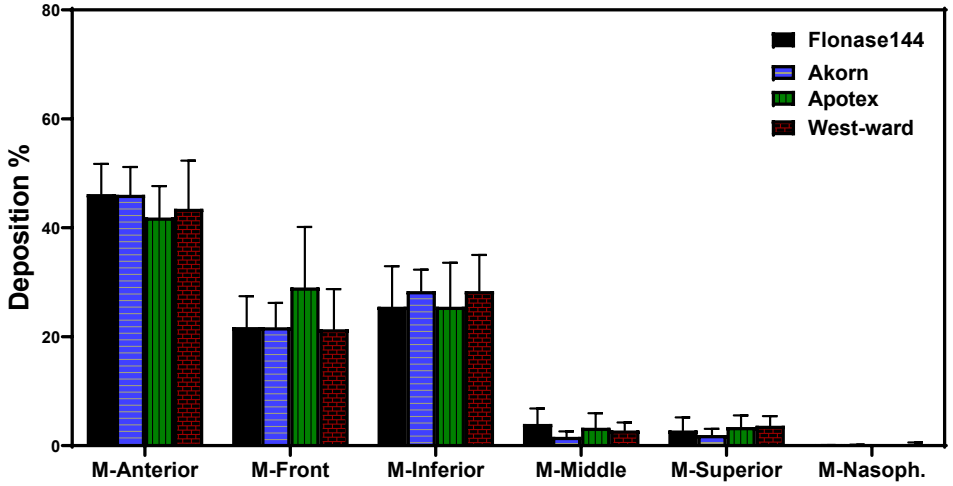


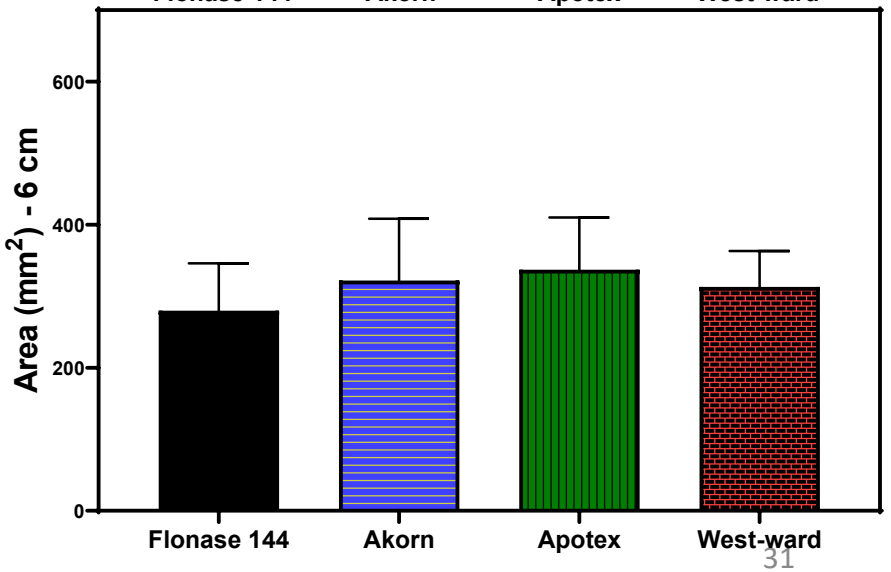
BE Test Metrics Vs. Regional Deposition: M Model

- M Model➔ No regional deposition differences unlike H model.



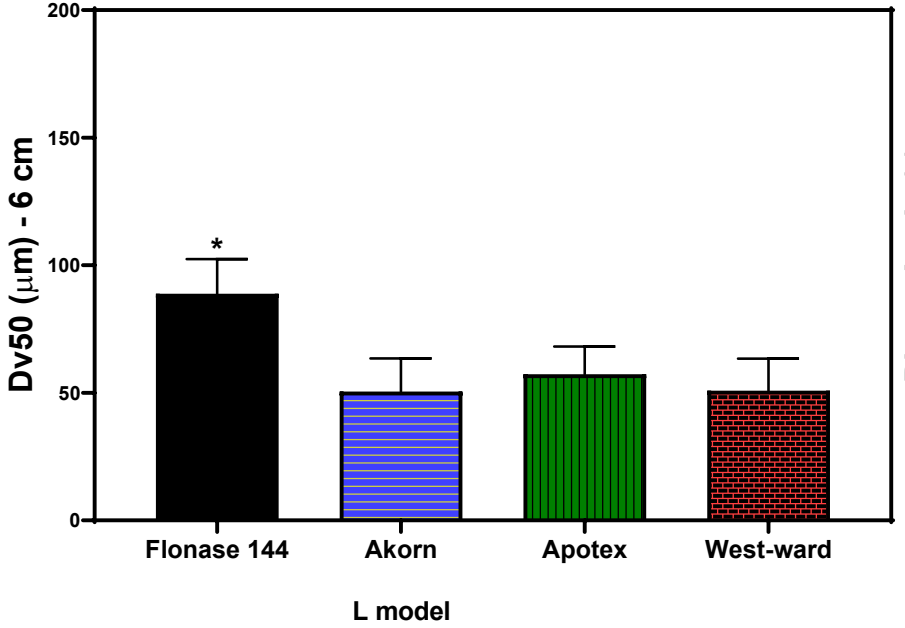


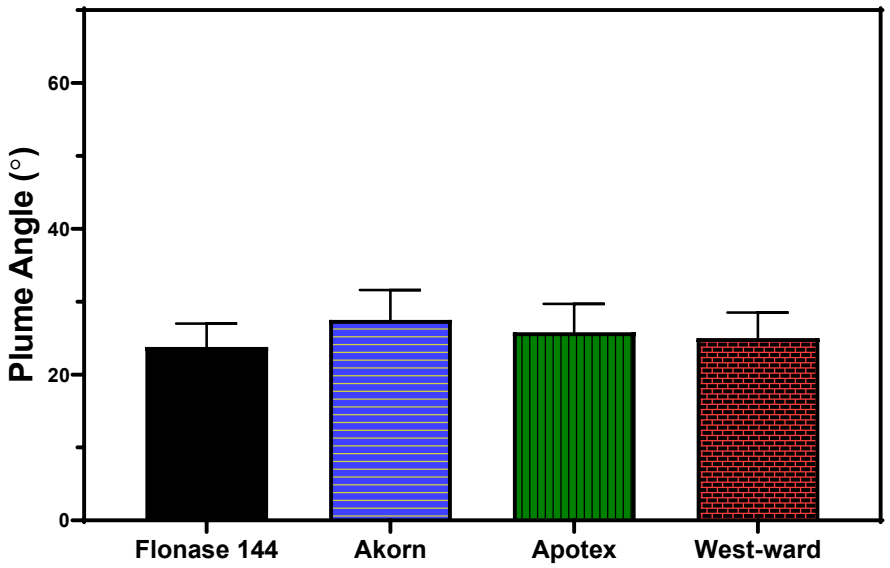


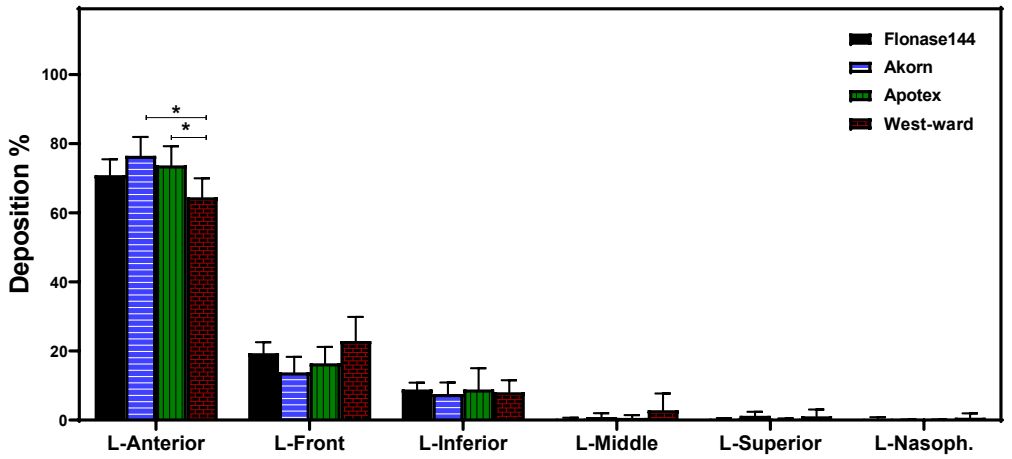


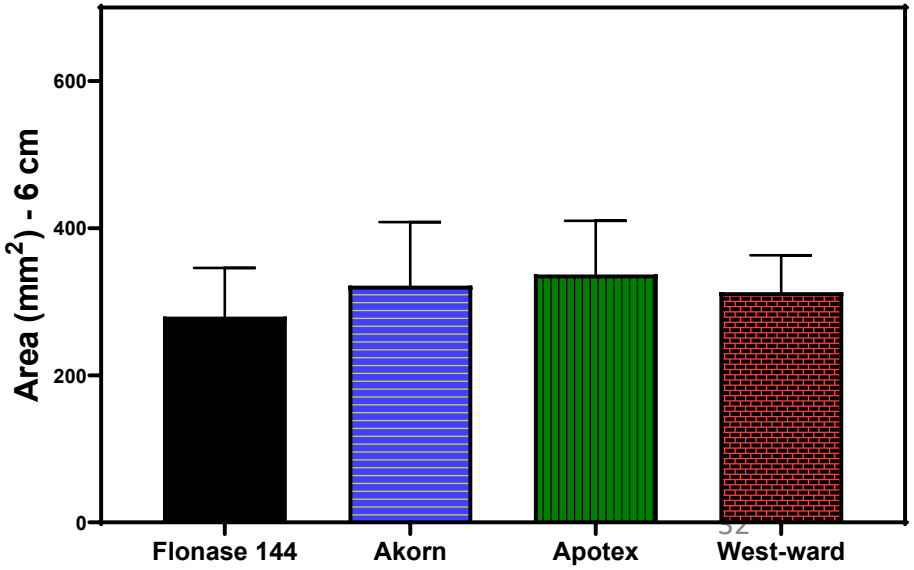
BE Test Metrics Vs. Regional Deposition: L Model

- L Model➔ No significant differences in posterior regions, but differences in anterior deposition.



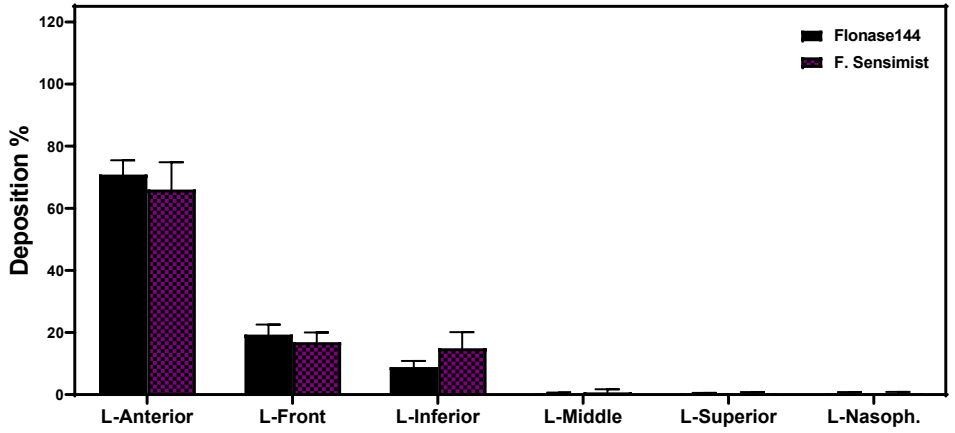


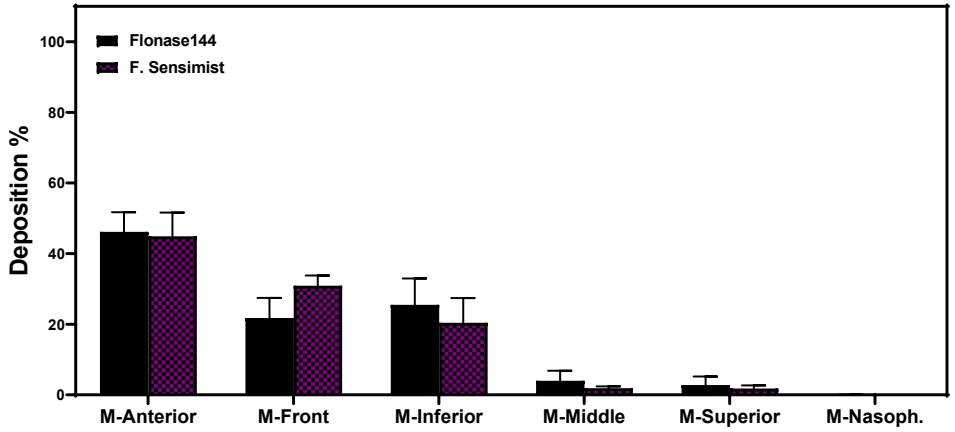


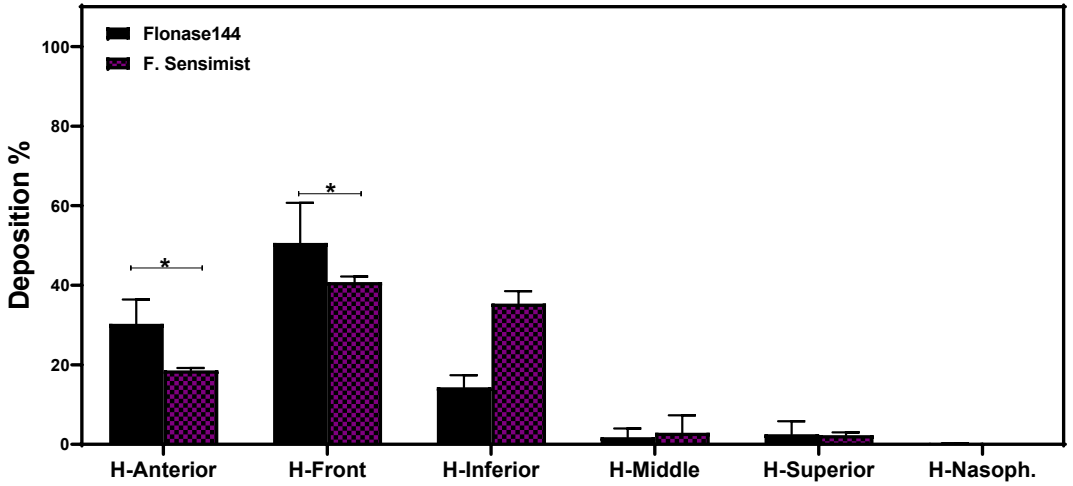


BE Test Metrics Vs. Regional Deposition- Sensimist (higher plume angle) vs F144 (higher DSD)

- T-test for each region of L, M, H models between F144 and Sensimist
- No significant difference in L and M models
- F144 vs FS in Front turbinate➔ significantly higher deposition for F144 in the H model again likely because of larger Dv50.







Plume Characterization at INV

- The data showed no significant differences in width between devices at INV.
- Significant difference was found between the area of Flonase 144 compared to Westward at the closest feasible distance to INV (10 mm vs at 5.6 mm).
- Westward showed the highest area and Flonase 144 showed the lowest area.

Table 1. The plume width of Flonase® 144 and the generic products (Akorn, Apotex and West-ward) measured at the INV of H model (5.6 mm) and the area of the same products at the closest distance possible to consider from the tip of the devices (10 mm), compared to the data at 3 cm and 6 cm based on n=9 from three different lot numbers (i.e., 3 units/per lot*3 lots*1 shots/unit).

Area (mm^2)		Flonase 144	Akorn	Apotex	West-ward
INV	Average	48.5	61.9	60.9	68.9
	SD	17.1	11.1	6.6	16.4
3 cm	Average	77.4	103.1	99.0	94.6
	SD	19.0	36.2	21.1	25.2
6 cm	Average	279.8	321.8	337.1	312.9
	SD	66.1	86.4	73.1	50.4
Width (mm)		Flonase 144	Akorn	Apotex	West-ward
INV	Average	6.6	7.6	6.7	6.7
	SD	0.8	0.9	0.8	1.0
6 cm	Average	25.4	30.0	27.6	26.6
	SD	3.5	4.4	4.3	3.9