Evaluating Topical Bioavailability In-Vivo: … Dermal Open Flow Microperfusion and Equivalence Testing by IVRT

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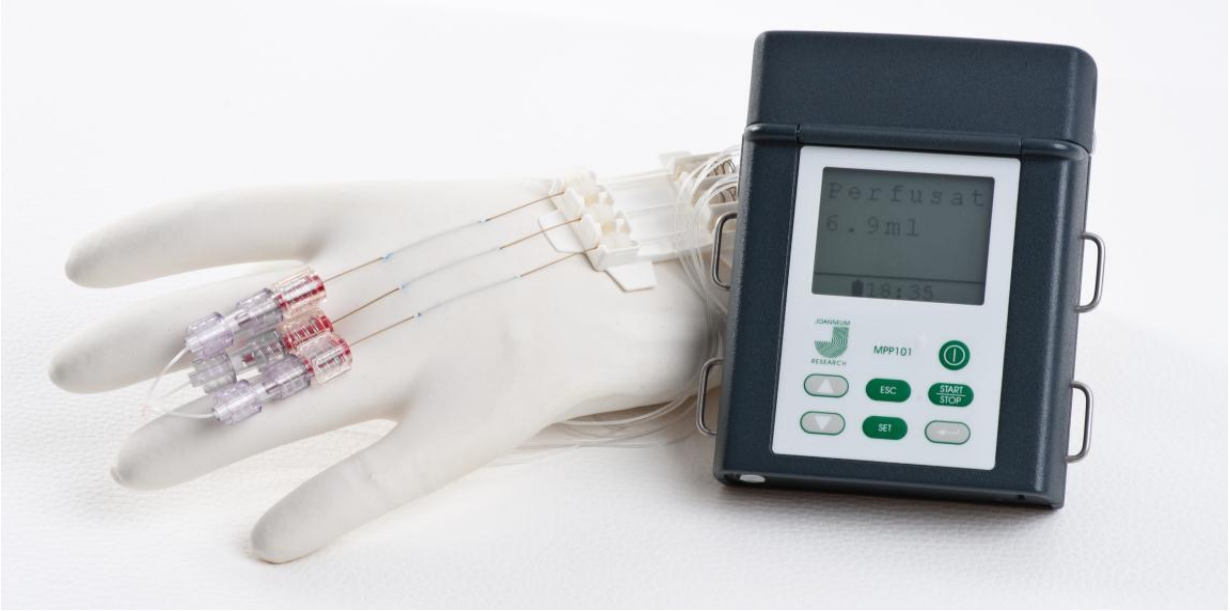
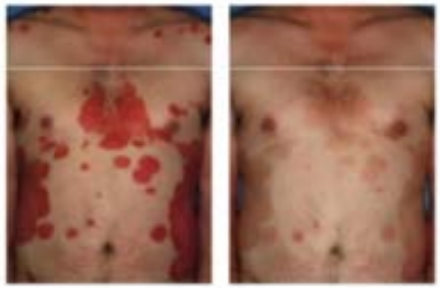


What dOFM Adds to Pharmacokinetics-Based BA Approaches

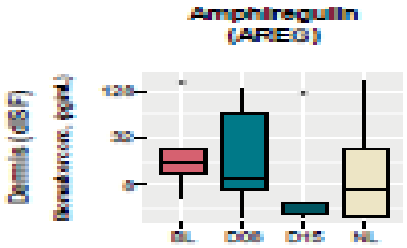
Predictive Bioavailability
The Big Picture

In-Vivo



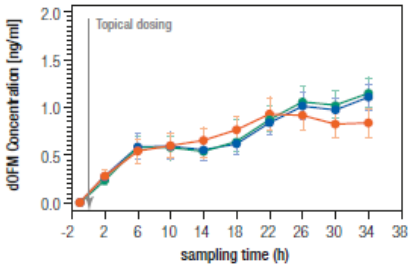
Endpoint Study

In-Vivo



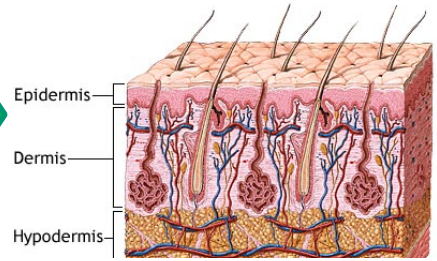
Biomarker Study

In-Vivo



PK Healthy Subjects

Ex-Vivo



Healthy Human Skin

In-Vitro

e.g. IVRT, IVPT

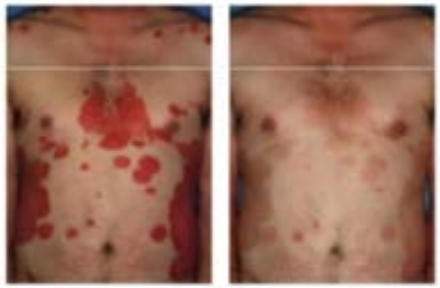
Physico-chem. Prop.

Q1 Q3

Q2

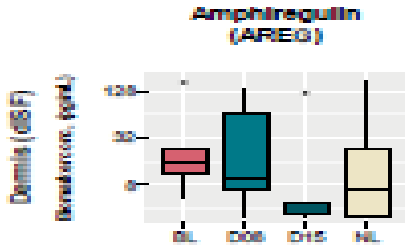
Predictive Bioavailability
The Big Picture

In-Vivo



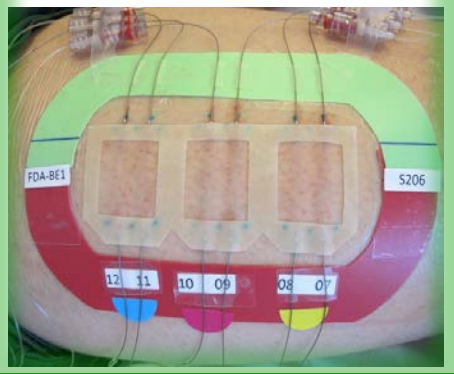
Endpoint Study

In-Vivo

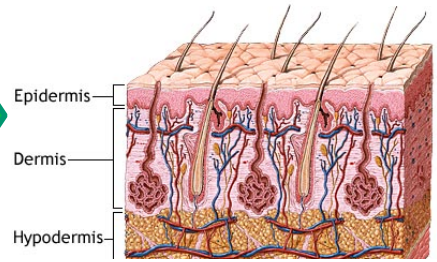


Biomarker Study

In-Vivo



Ex-Vivo



Healthy Human Skin

In-Vitro

e.g. IVRT, IVPT

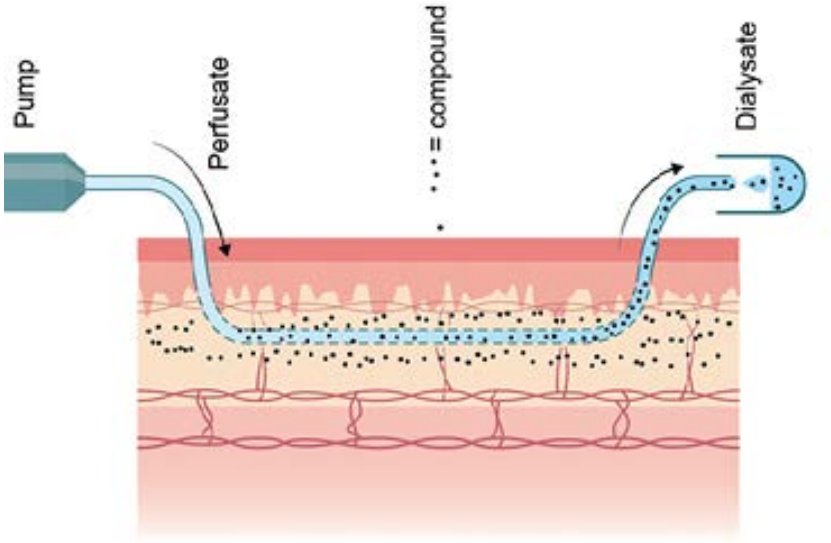
Physico-chem. Prop.

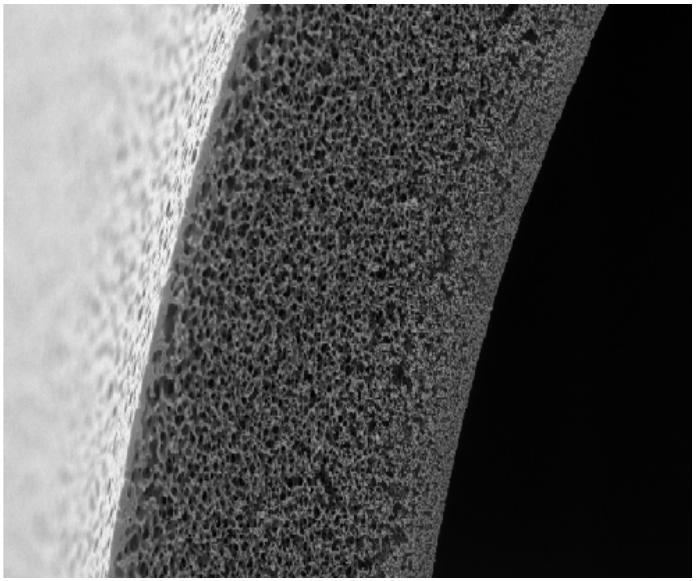
Q1 Q3

Q2

Pharmacokinetics-Based BA Approaches
Dermal Microdialysis (dermal MD)

✓ **MD samples represent diluted and filtered interstitial fluid**





dMD has been used for topical BA:

Benfeldt *JID* 2007 (Lidocaine, 5 h)
Tettey-Amlalo *EurJPharmSci* 2009 (Ketoprofen, 5 h)
Incecayir *PharmRes* 2011 (Oxytetracycline, 4 h)
García Ortiz *SkinPharmPhysiol.* 2011 (Metronidazole, 5 h)

Why is dermal MD not accepted by FDA today?

Strengths

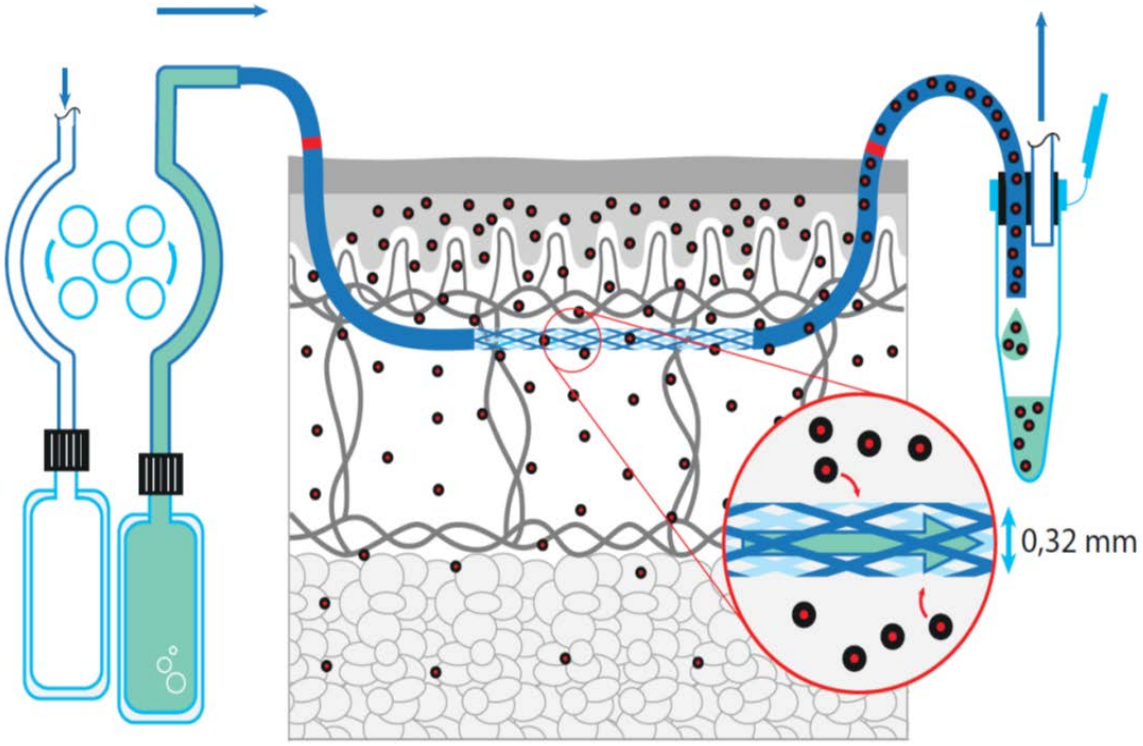
1. Provides a direct in-vivo measurement of the rate and extent of the active moiety at or near the site of action in the skin.
2. Evidence indicates that dermal MD has the potential to differentiate pharmacokinetic profiles by their magnitude.

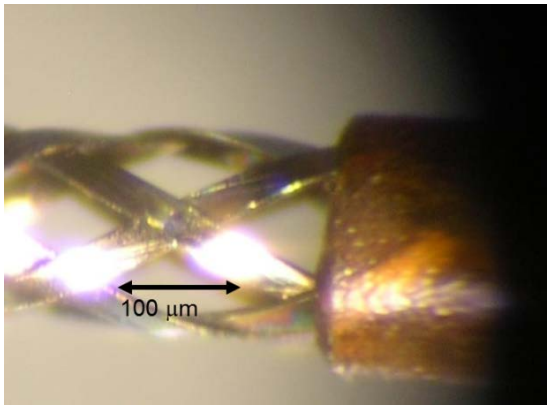
Limitations

1. Limitations linked to membrane, e.g. pore size and adsorption
2. Limited sampling time, often < 8 hours
3. Various factors contribute to data variability

Open Flow Microperfusion

✓ **OFM samples represent <u>diluted but unfiltered</u> interstitial fluid**

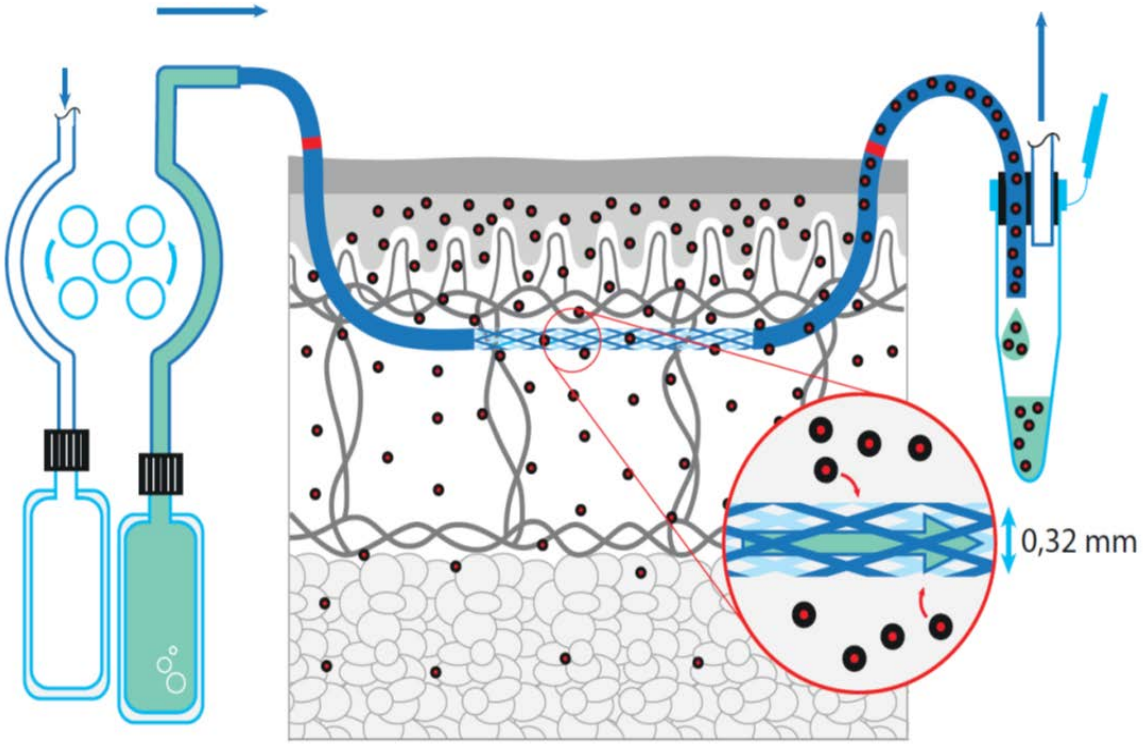


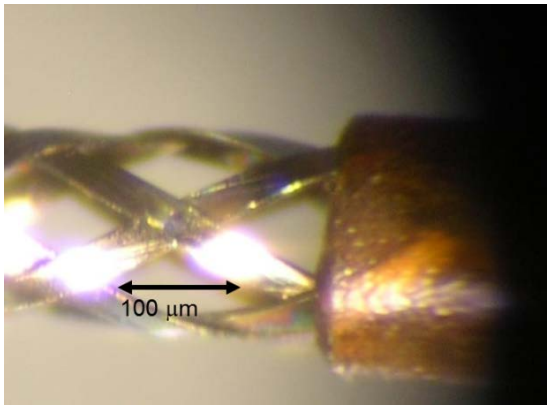


CE-certified for clinical use

Open Flow Microperfusion

✓ **Limitation 1 solved: all drugs are accessible in-vivo in the dermis**

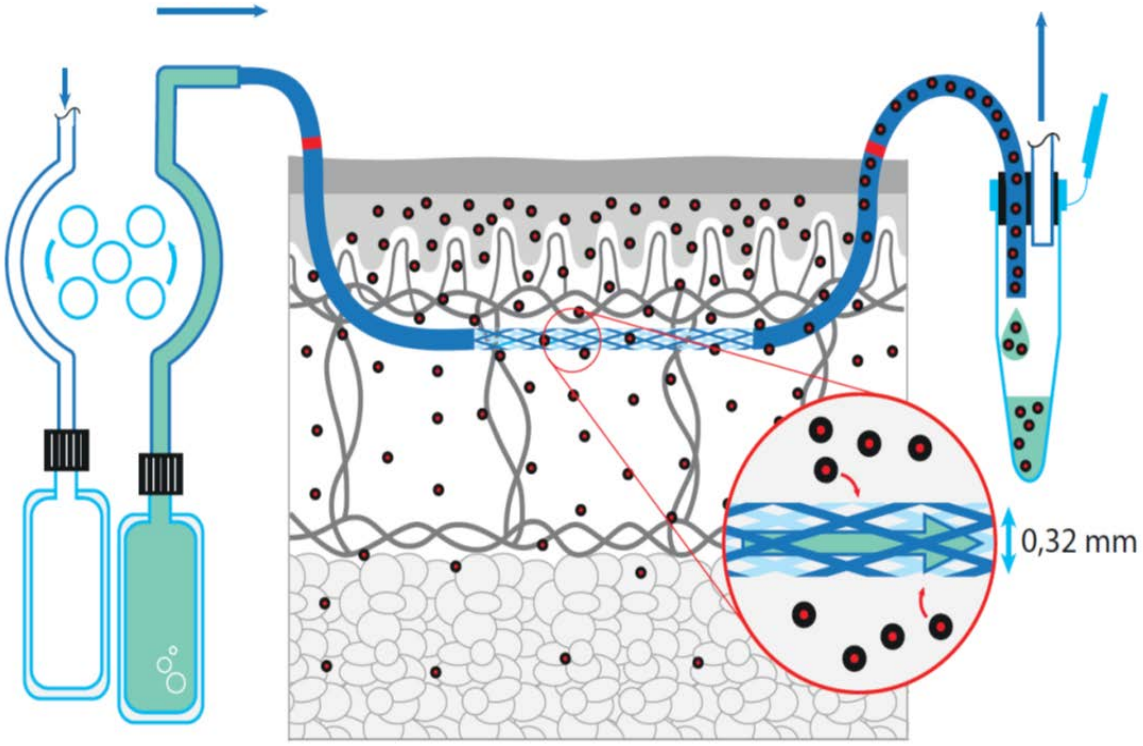


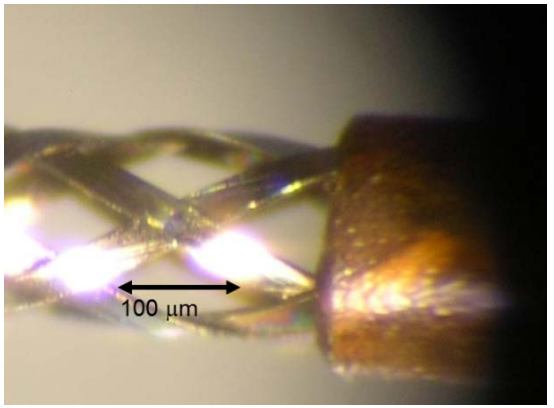


CE-certified for clinical use

Open Flow Microperfusion

✓ **Limitation 2 solved: In-vivo sampling in the dermis up to 48 hours**



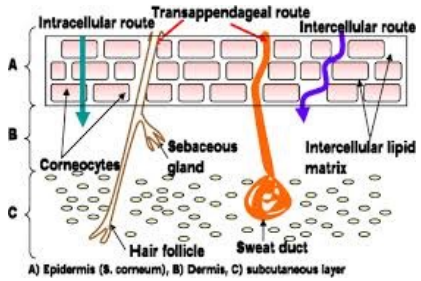


dOFM used for PK-PD in skin:

Acyclovir (topical) – 36 h clinical
Corticoid (topical) – 26 h clinical
Antibody (SC) – 17 h clinical
Acyclovir (topical) – 36h ex-vivo human skin
NCE (topical) – 24 h ex-vivo human skin

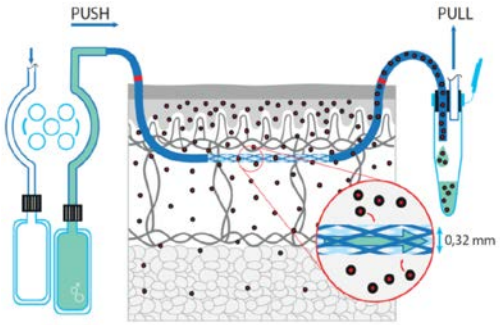
Continuous dermal ISF sampling
Sources of Variability

variability due to sampling site



- Differences in skin structure
  - Between subjects
  - Parts of the body
- Hairiness
- Sweat ducts
- Day/night rhythm of local blood flow
- Hair shaving
- Skin care products use
- Skin condition (e.g. solarium)

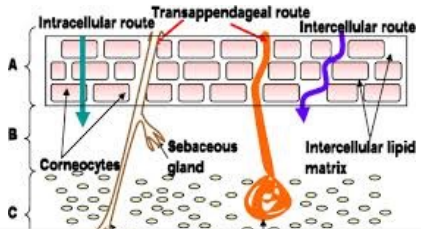
variability due to methods



- Trauma formation (OFM/MD)
- Dosage application
- Probe depth (OFM/MD)
- Flow rate (OFM/MD)
- Local blood flow (OFM/MD)
- Lateral diffusion
- Systemic diffusion
- Room temperature and humidity

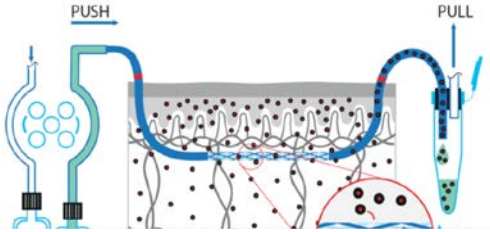
Continuous dermal ISF sampling
Sources of Data Variability

variability due to sampling site



- Between subjects
  - Parts of the body
- Hairiness
- Sweat duct
- Day/night rhythm of local blood flow
- Hair shaving
- Skin care products use
- Skin condition (e.g. solarium)

variability due to methods



- Dosage application
- Probe depth (dOFM)
- Flow rate (dOFM)
- Local blood flow
- Lateral diffusion
- Systemic diffusion
- Room temperature and humidity

➔ **control all significantly contributing factors that add to data variability**

➔ **factors that cannot be controlled are monitored**

dOFM
(1) Apparatus Qualification

New dOFM probe

- 0.5 x 15 mm sampling mesh
- patent granted
- use of up to 48 hours

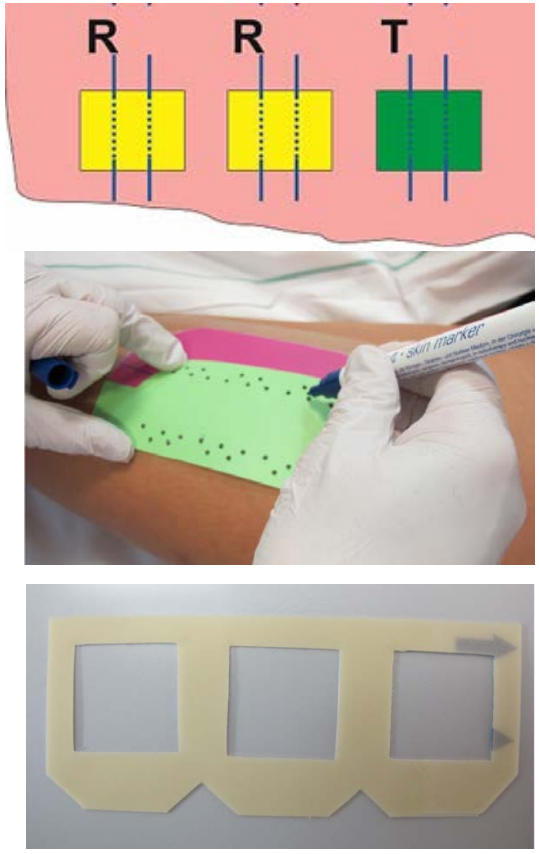
dOFM pump

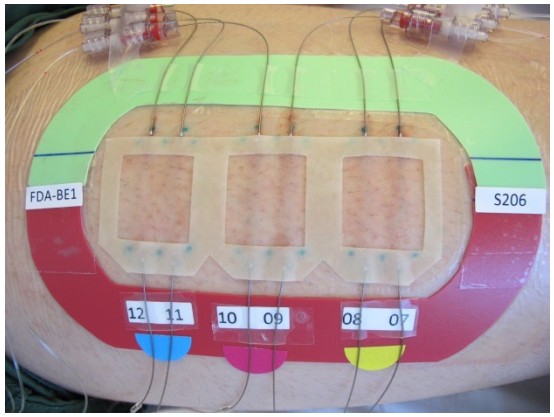
- portable
- 0.1 – 10 µl/min
- Sterile fluidic kit
- operates 3 OFM probes

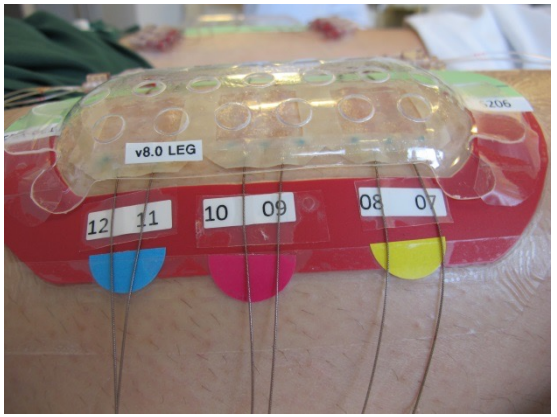
CE certified for clinical use

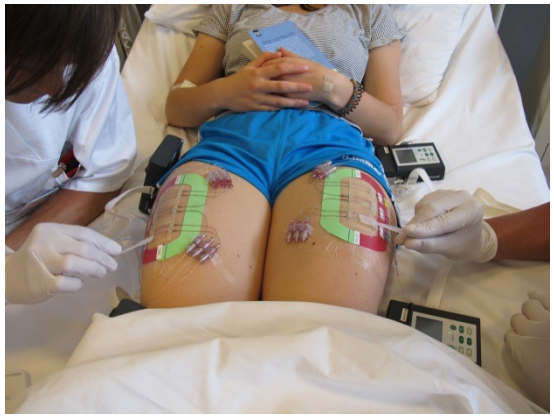
dOFM
(2) Performance Optimization

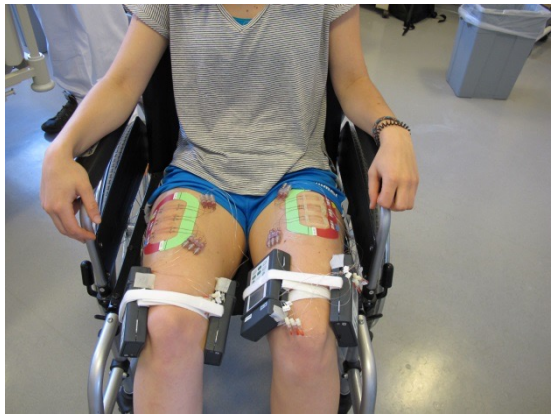
✓ **All dOFM procedures are highly standardized**





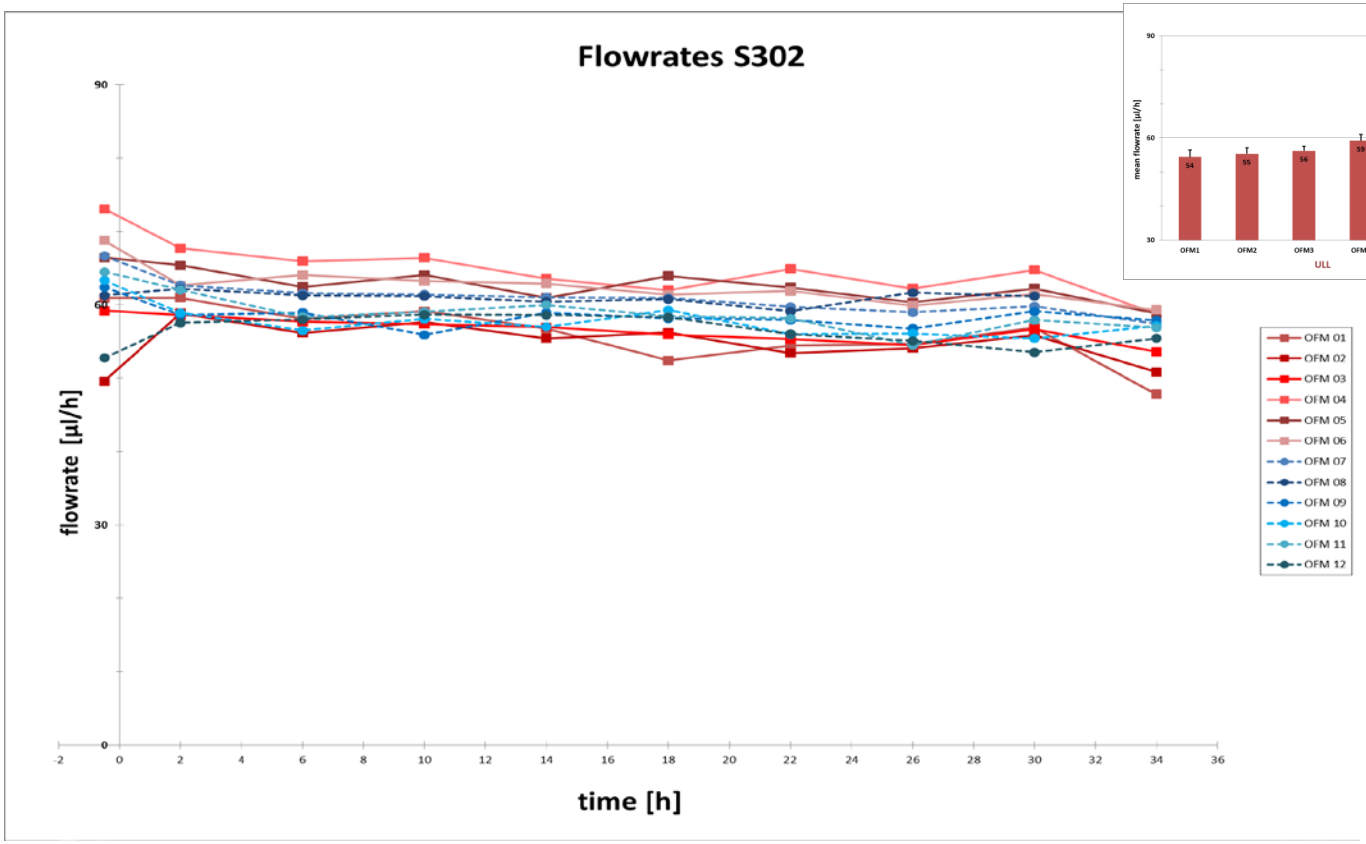


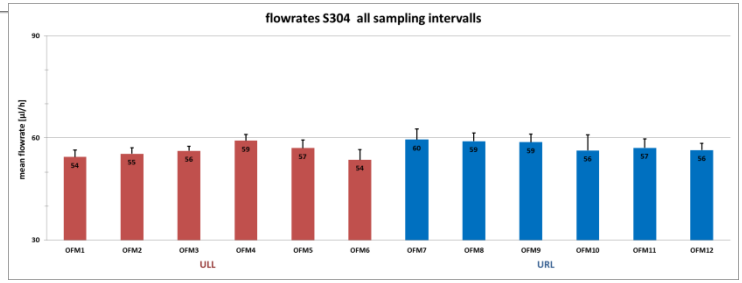


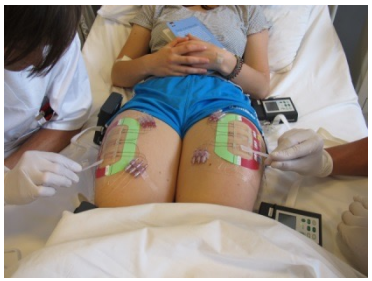


dOFM
(3) Performance Verification

✓ **dOFM provides a stable flow rate for 36 hours**

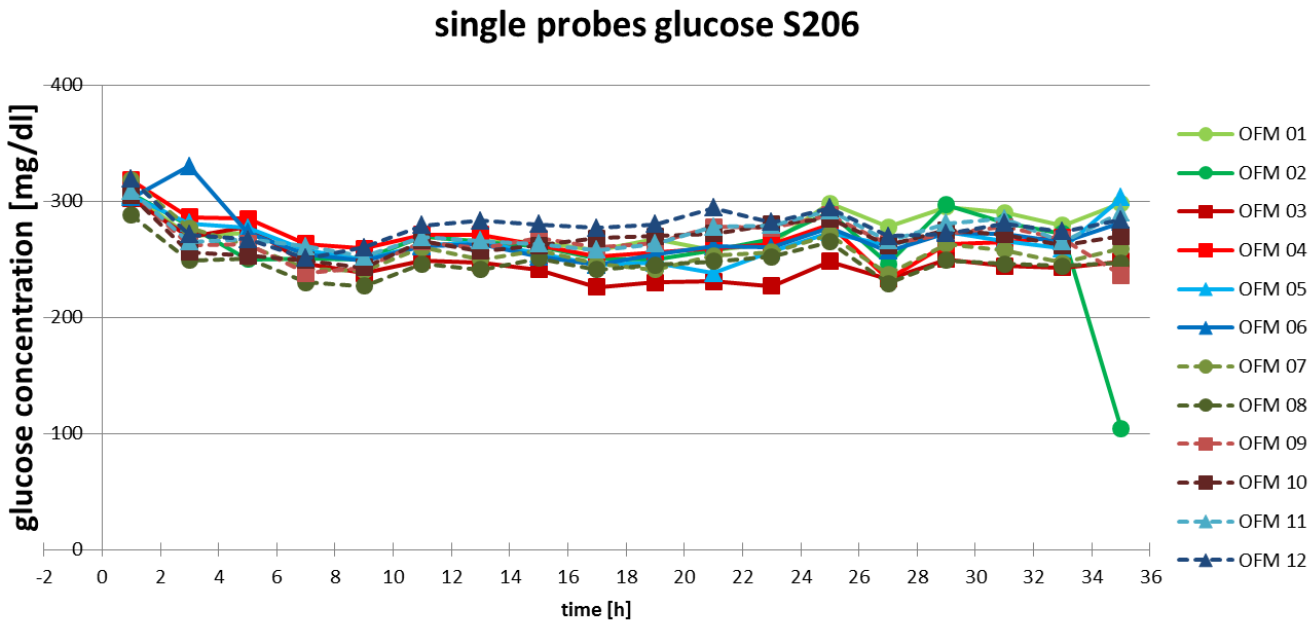


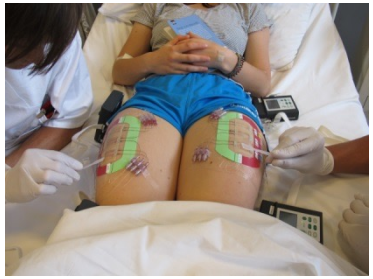




dOFM
(3) Performance Verification

✓ **dOFM is used to sample analytes for 36 hours**

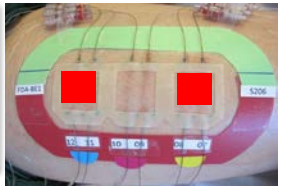




Method Validation for Acyclovir

Test for Systemic Exposure

- ✓ **No systemic exposure**
- ✓ **No influence on PK at dOFM site**



50 mg/cm² US Zovirax

$$R = \frac{\#Blood\ Samples > LLOD}{\#Total\ Blood\ Samples}$$

- no systemic exposure if R<0.05

Results

	min	median	P90	P95	P99	max
R	0	0.013	0.256	0.039	0.051	0.064

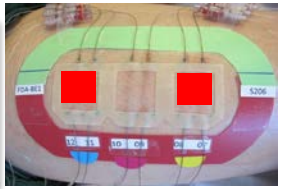
Methodology

- 6 subjects
- 10,000 bootstrap estimates
- Confidence interval created for true population value of test statistic R
- One-sided 95% confidence interval

Method Validation for Acyclovir

Test for Lateral Diffusion

- ✓ **Negligible lateral diffusion in a few cases after 24 h**
- ✓ **No significant influence on PK at adjacent dOFM sites**



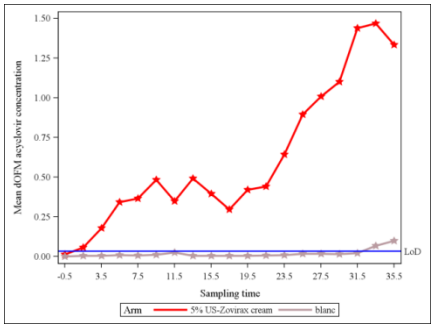
50 mg/cm² US Zovirax

- $R = \frac{\#dOFM\ BLANC\ Sites > LLOD}{\#dOFM\ Samples\ ZOVIRAX\ US\ Sites > LLOD}$
- no lateral diffusion if $R < 0.05$

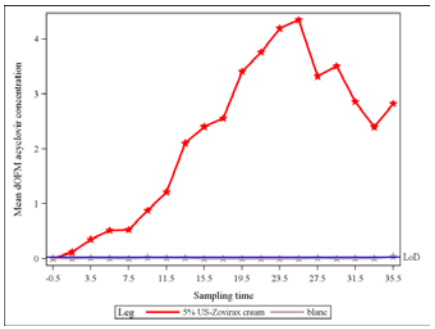
Results

	min	median	P90	P95	P99	max
R	0.008	0.076	0.109	0.118	0.135	0.183

PK profile arms (n=6)



PK profile legs (n=6)

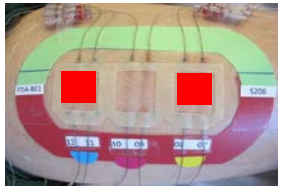


Methodology

- 6 subjects
- 10,000 bootstrap estimates
- Confidence interval created for true population value of test statistic R
- One-sided 95% confidence interval

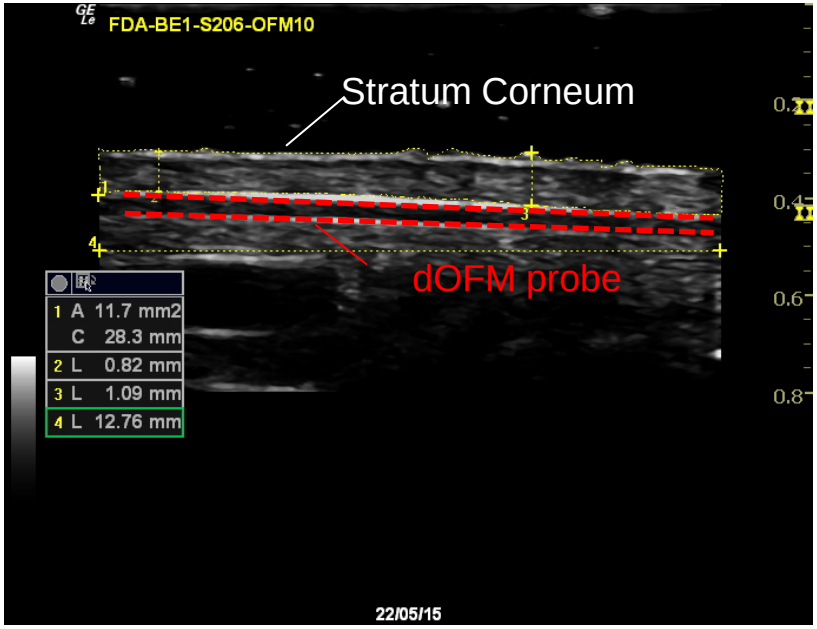
Method Validation for Acyclovir dOFM Probe Depth

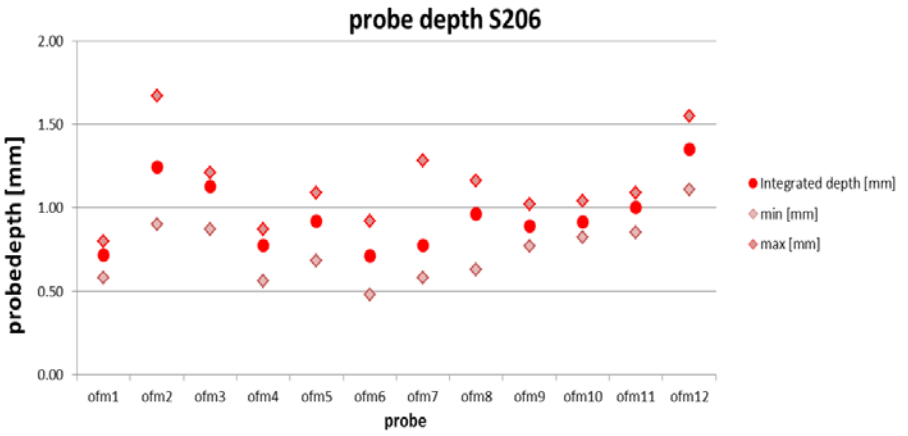
✓ **Uniform probe depth**



50 mg/cm² US Zovirax

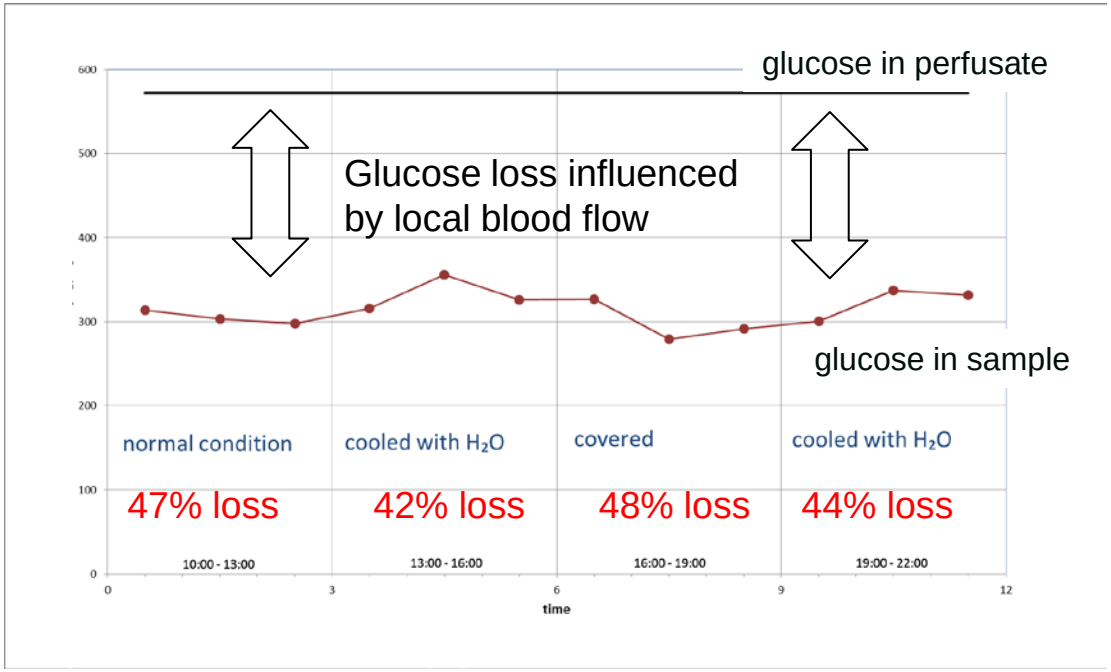
Monitoring of probe depth along the whole exchange area





dOFM Method Validation
Local Blood Flow

✓ **Local blood flow monitoring**



Glucose was used as an internal standard in OFM perfusate

Cooling was used to

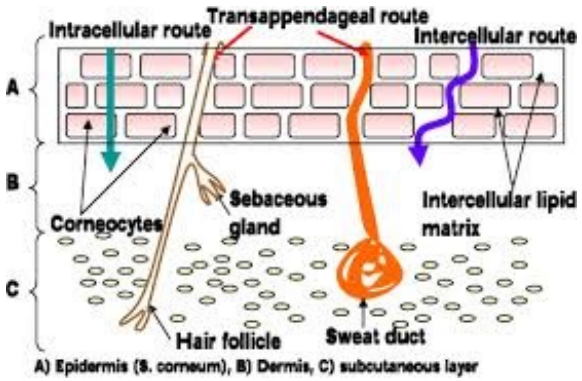
- reduce local blood flow
- lower glucose loss from perfusate

6 dOFM probes in one subject

dOFM
Controlled and Monitored Factors

variability due to sampling site

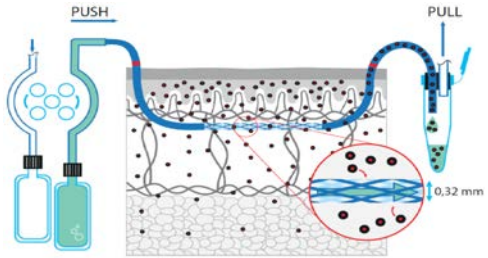
• Hairiness	➔	not controlled
• Hair shaving	➔	subjects are shaved 5 days before dOFM visit
• Sweat ducts	➔	not controlled
• Skin permeation behaviour	➔	monitored by TEWL and impedance
• Skin products use	➔	not allowed 5 days before dOFM visit
• Skin condition (e.g. Solarium)	➔	visual check at screening visit



dOFM
Controlled and Monitored Factors

✓ **Limitation 3 solved: In-vivo variation significantly reduced**

	variability due to methods
Controlled by cooling	← Trauma formation (OFM/MD)
Controlled by application template	← Application site
Controlled by standardization	← Dosage application
Monitored by ultrasound	← Probe depth (OFM/MD)
Monitored by sample weight	← Flow rate (OFM/MD)
Monitored by glucose marker	← Local blood flow (OFM/MD)
Negligible	← Lateral diffusion
No systemic exposure	← Systemic diffusion
Controlled 22 ± 1°C & 40 - 60% RH	← Room temperature & relative humidity



dOFM
Quality Controlled Workflow

✓ **All dOFM procedures are highly standardized**

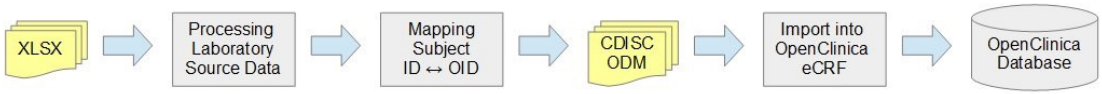
GCP

eCRFs
SDFs

GLP lab

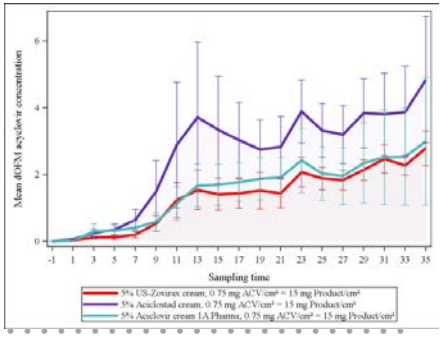
Method Validation Plan
Method Validation Report
Method SOPs
Study Analysis Plan

Data Management Plan



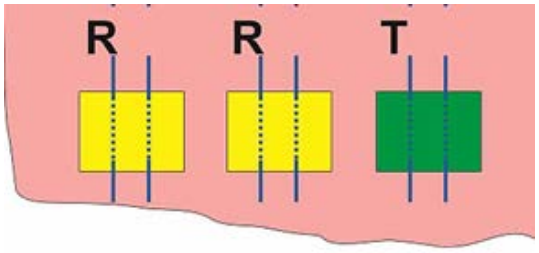
Software Verification and Validation Report
Software Verification and Validation Report
OFMLabData Import Validation Plan
OFMLabData Import Validation Report
OFMLabData Import SPOs

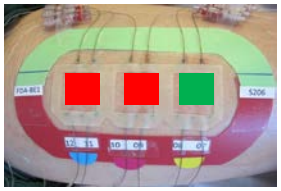
Statistical
Analysis
Plan

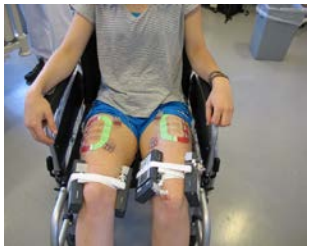


Clinical Bioavailability
dOFM Study Approach

- Clinical study in healthy subjects (n=20)
- ***R****eference*: Zovirax cream 5% (US)
- ***T****est*: Aciclovir 1A Pharma Cream 5% (Austria)
- Aims:
  - Investigate BA for *R* vs *R* for 36 h post-dose
  - Investigate BA for *T* vs *R* for 36 h post-dose

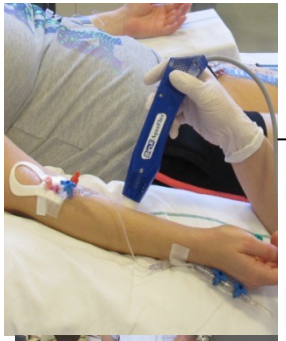


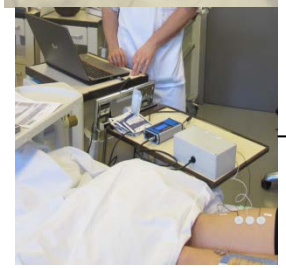


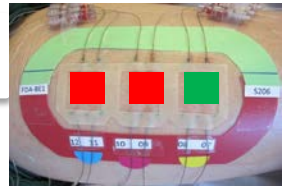


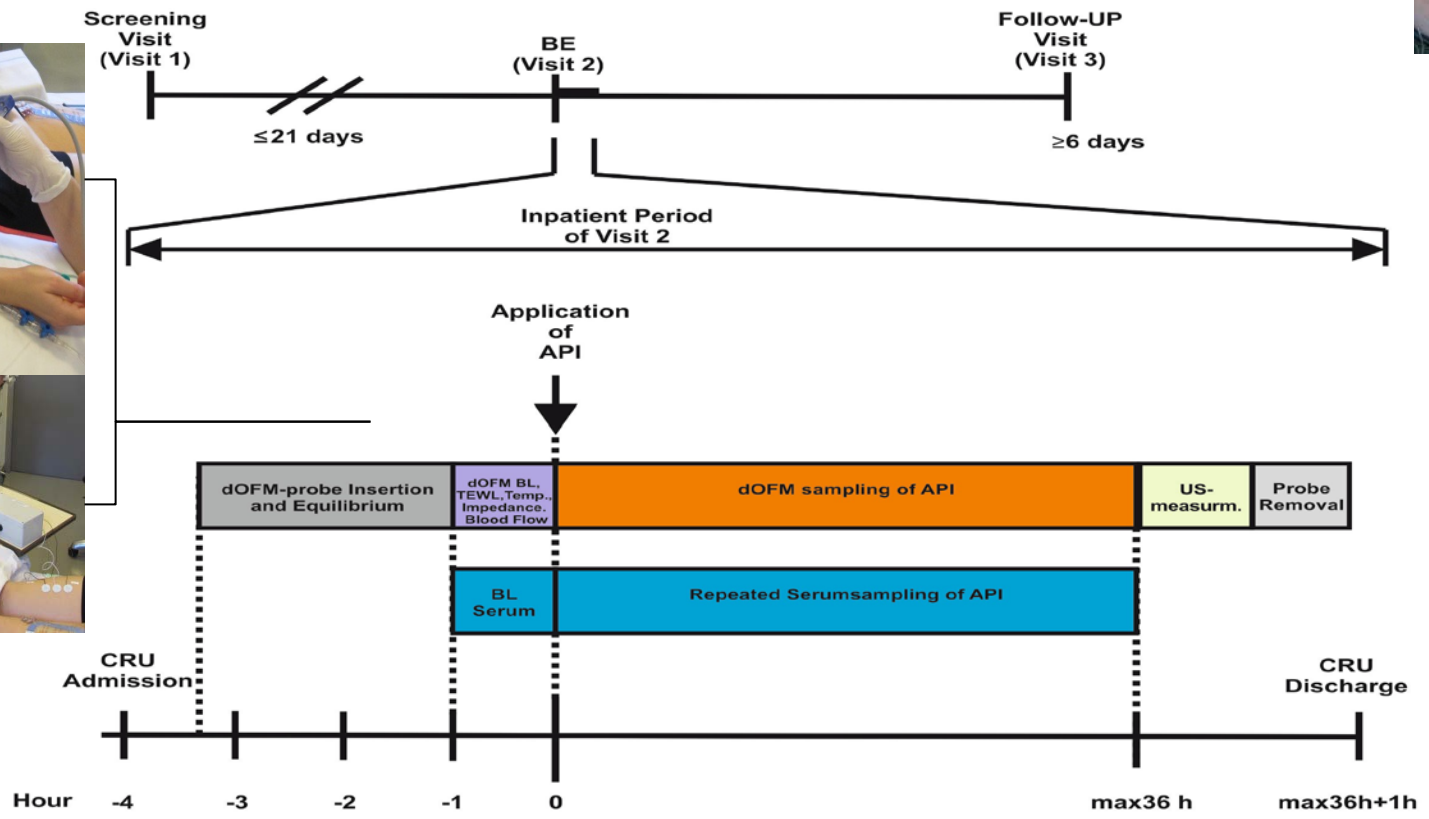
Clinical Bioavailability
Clinical BA Set-Up

✓ **SOP controlled clinical BA protocol**



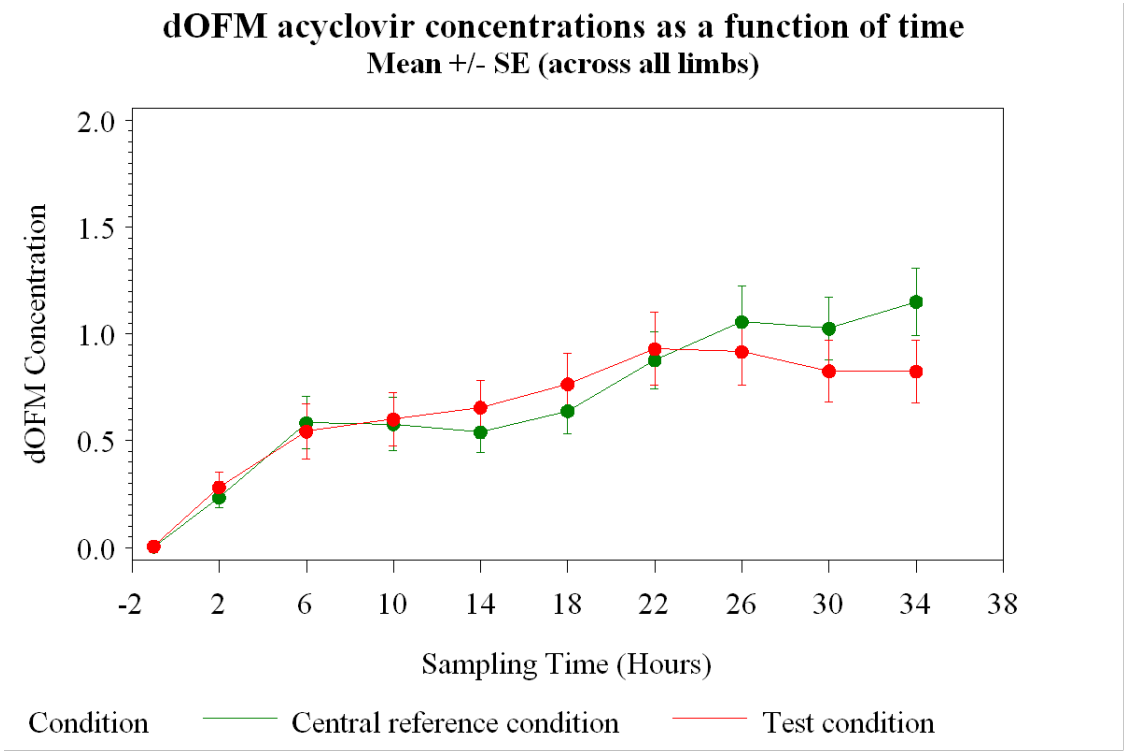






Clinical Bioavailability
Test versus Reference

✓ **Bioavailability: AUC and T_{max} of Aciclovir A1 are highly reproducible**
AUC and T_{max} of Zovirax US are highly reproducible



Clinical Bioavailability
Test versus Reference

✓ **Bioavailability: BA is different for Aciclovir A1 vs Zovirax US based on AUC**
BA is different for Aciclovir A1 vs Zovirax US based on C_{max}

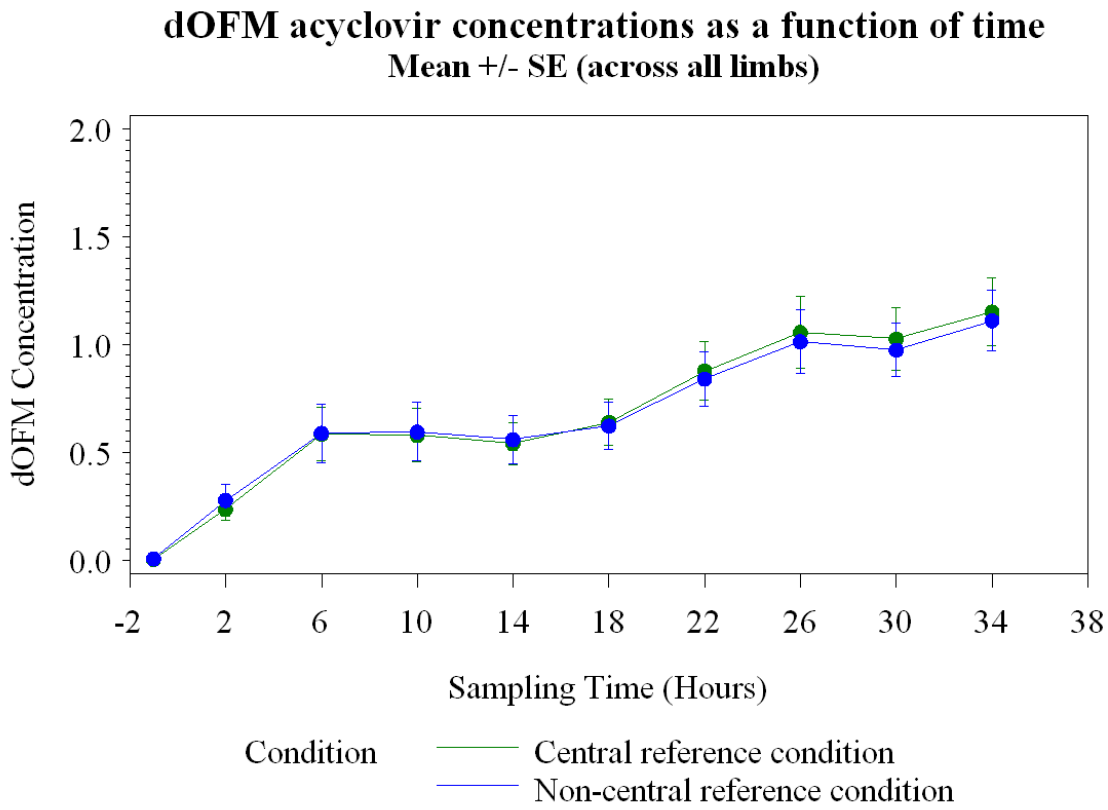
Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.369 ; 0.050] or [69.1 % ; 105.2 %]	[-0.223 ; 0.223] or [80% ; 125%]	x Failed
log(C_{max})	[-0.498 ; 0.022] or [60.8 % ; 102.2%]		x Failed

BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between test and reference condition

BA is established if $CI_{90\%}$ falls within the limits of log(0.8)=-0.223 and log(1.25)=0.223 (cf. FDA Guidance For Industry)

Clinical Bioavailability
Reference versus Reference

✓ **Bioavailability: AUC and C_{max} of Zoriax US are highly reproducible**



Clinical Bioavailability
Reference versus Reference

✓ **Bioavailability: Same BA for Zovirax US vs Zovirax US based on AUC**
Same BA for Zovirax US vs Zovirax US based on C_{max}

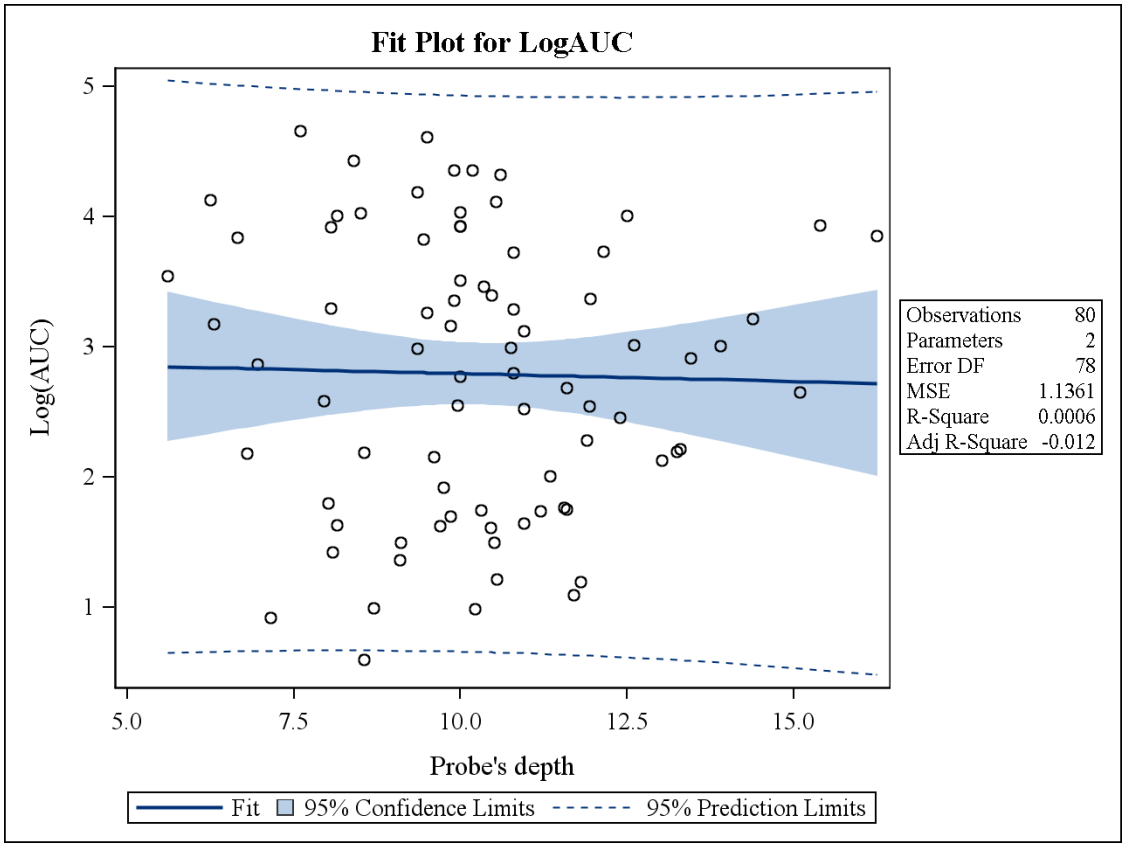
Outcome variable	$CI_{90\%}$	BE-limits	$CI_{90\%}$ within BE-limits
log(AUC0-36h)	[-0.148 ; 0.162] or [86.2 % ; 117.5 %]	[-0.223 ; 0.223] or [80% ; 125%]	**passed**
log(C_{max})	[-0.155 ; 0.190] or [85.7 % ; 120.9%]		**passed**

BA is tested for the difference of the log-transformed outcome variables (AUC, C_{max}) between the two reference conditions

BA is established if $CI_{90\%}$ falls within the limits of log(0.8)=-0.223 and log(1.25)=0.223 (cf. FDA Guidance For Industry)

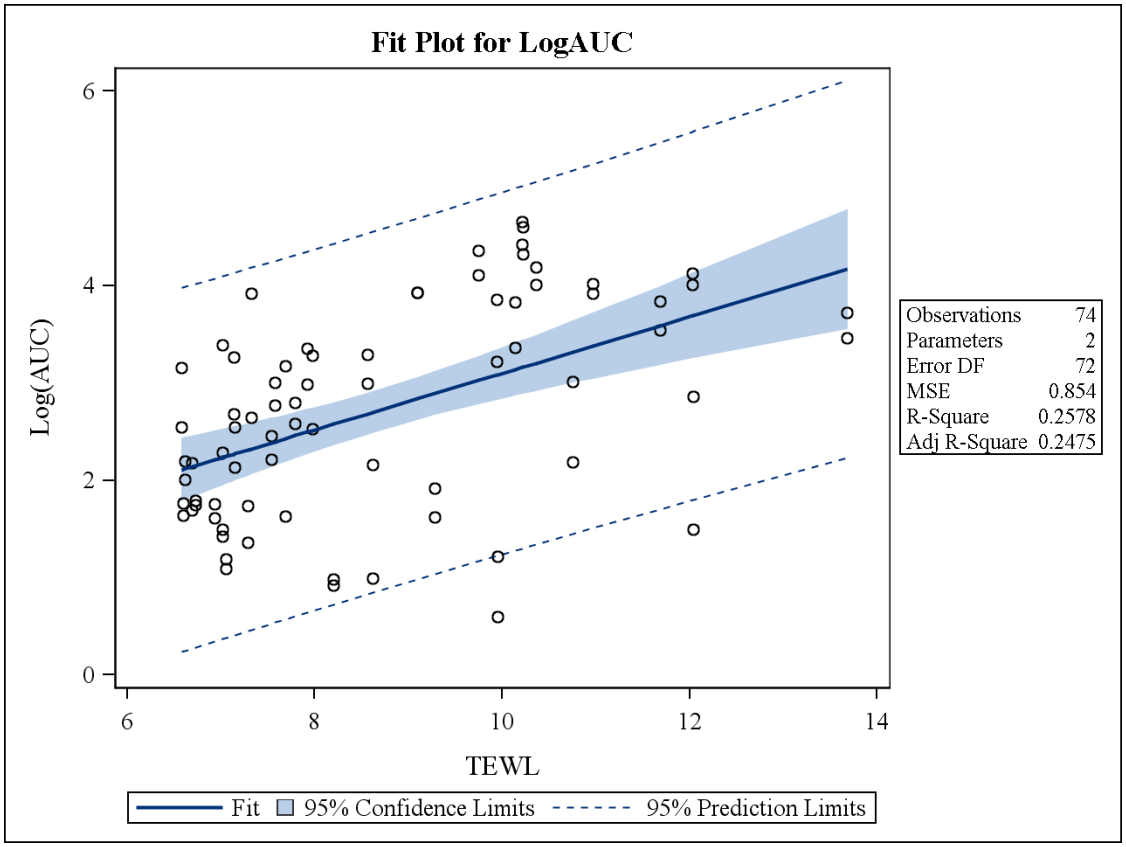
Clinical Bioavailability
Influence of Probe Depth on AUC of Acyclovir

✓ **dOFM acyclovir concentration does not correlate with probe depth**



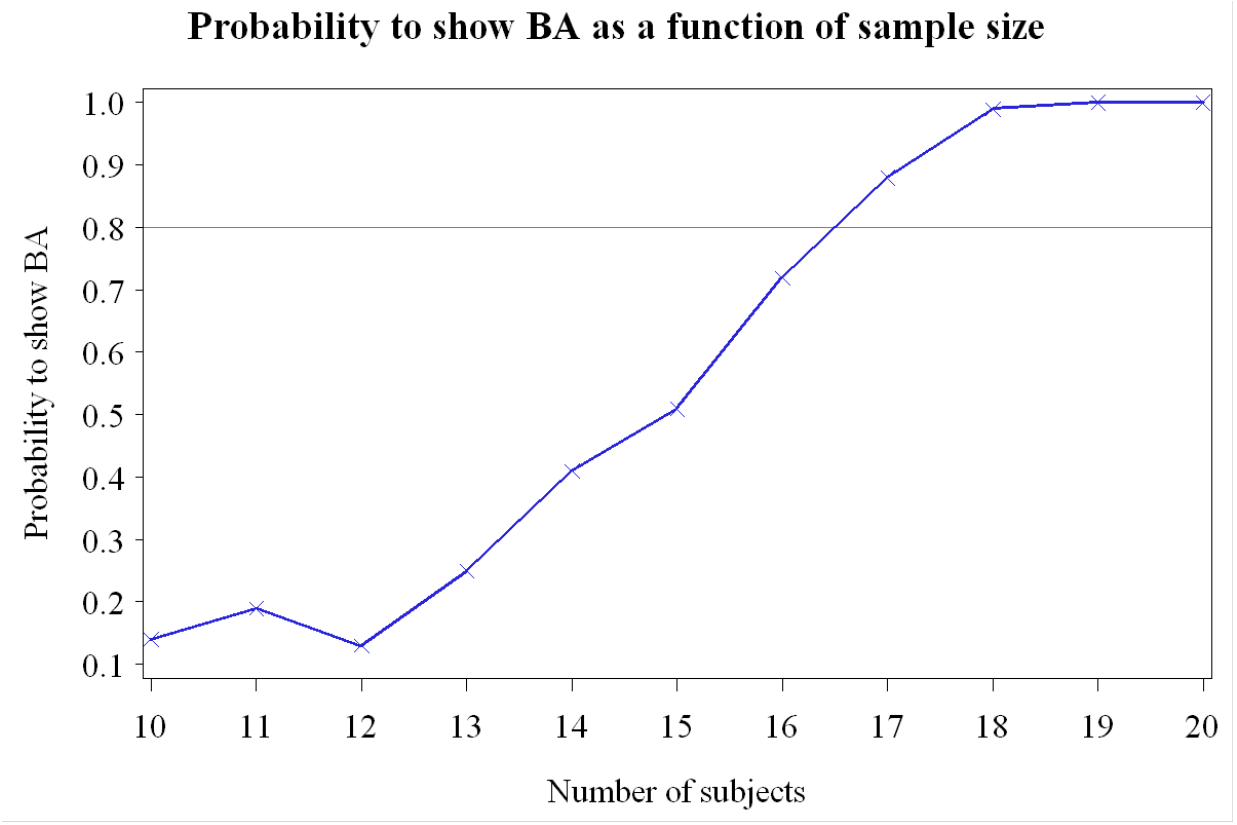
Clinical Bioavailability
Correlation of TEWL with AUC of Acyclovir

✓ **dOFM acyclovir concentration correlates with TEWL**



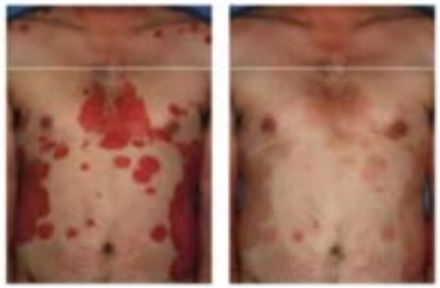
Clinical Bioavailability
Power Calculation to Show BA

✓ **17 subjects are sufficient to show BA for acyclovir when using dOFM**



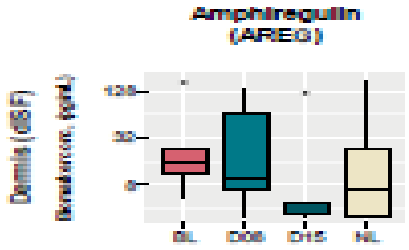
Predictive Bioavailability
The Big Picture

In-Vivo



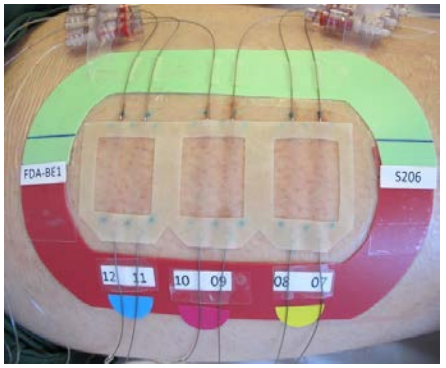
Endpoint Study

In-Vivo

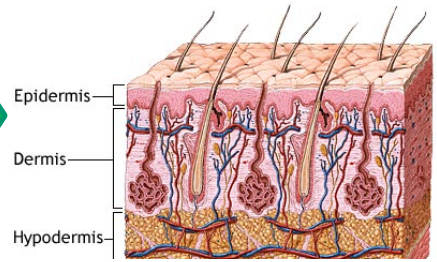


Biomarker Study

In-Vivo



Ex-Vivo



Healthy Human Skin

In-Vitro

e.g. IVRT, IVPT

Physico-chem. Prop.

Q1 Q3

Q2

IVRT
Method Description

- Apparatus: Hanson vertical diffusion cells (VDC, volume: 12 mL, orifice: 15 mm)
- Receptor medium: 0.9% saline solution (degassed)
- Sampling: 0.5, 1, 2, 3, 4, 5 and 6 hours after dosing
- Membrane: Tuffryn® membrane (25 mm, 0.45 µm)
- Stirring speed: 600 rpm
- Temperature: 32˚C

IVRT
(1) Apparatus Qualification

✓ **Successful qualification of laboratory and IVRT apparatus**

Results

Test	Pass
P1: Environmental conditions	✓
P2: Capacity of the cells	✗
P3: Diameter of the orifice of the cell	✓
P4: Temperature of the receptor medium	✓
P5: Speed of the magnetic stirrer	✓
P6: Dispensed sampling volume	✓

9.77 mL instead of 12 mL

Methodology

Test of VDC apparatus for consistent operation within established limits and tolerances.

IVRT
(2) Performance Verification

✓ **Successful performance verification**

Methodology

- IVRT study was conducted according to the USP general chapter <1724>
- 1% hydrocortisone cream (BP 1% w/w; LycorTM 1%, Micro Labs Limited, Bangalore, India)

Results

✓ **Perfect sink conditions:** Acyclovir solubility > 10 times maximum receptor medium conc. observed during the IVRT study

✓ R^2 values range were [0.95 - 1] for all calculated **release rates** >0.9

✓ Blank samples before start from each cell showed **no** acyclovir **carry over**

✓ The **inter-run CV** (12.7%) and **intra-run CV** (6.8-10.2%) < 15%

✓ x_8 = 1.04 and x_{29} = 1.32 **meet** the 75% - 133.33% limits of the **USP general chapter <1724>**

IVRT
(3) Method Validation for Acyclovir

- ✓ **IVRT was successfully validated for acyclovir**
- ✓ **Tests for selectivity, sensitivity and specificity are ongoing**

Results

- ✓ **Membrane inertness**: Recovery of 105.5%
- ✓ **Receptor solubility test**: Solubility > 10 times maximum receptor medium conc. observed
- ✓ **Linearity**: Lowest R^2 value was 0.97, no outlier
- ✓ **Precision:** Inter-run variability 5.8 %; intra-run variability 4.4 %
- ✓ **Recovery:** < 10%, i.e. no excessive acyclovir depletion
- ✓ **Robustness:** Release rate for temperature and stirring speed variation deviate < 15%
- ✗ **Sensitivity, specificity and selectivity:** ongoing

Methodology

- IVRT study was conducted according to the USP general chapter <1724> and HPLC-UV method validation according to ICH Q2
- Validation of the IVRT method for acyclovir (Zovirax cream 5% - GSK, AT)

IVRT
Comparative Study

✓ **All test products were non-bioequivalent relative to Zovirax US**
✓ **Zovirax US was bioequivalent to itself**

Results

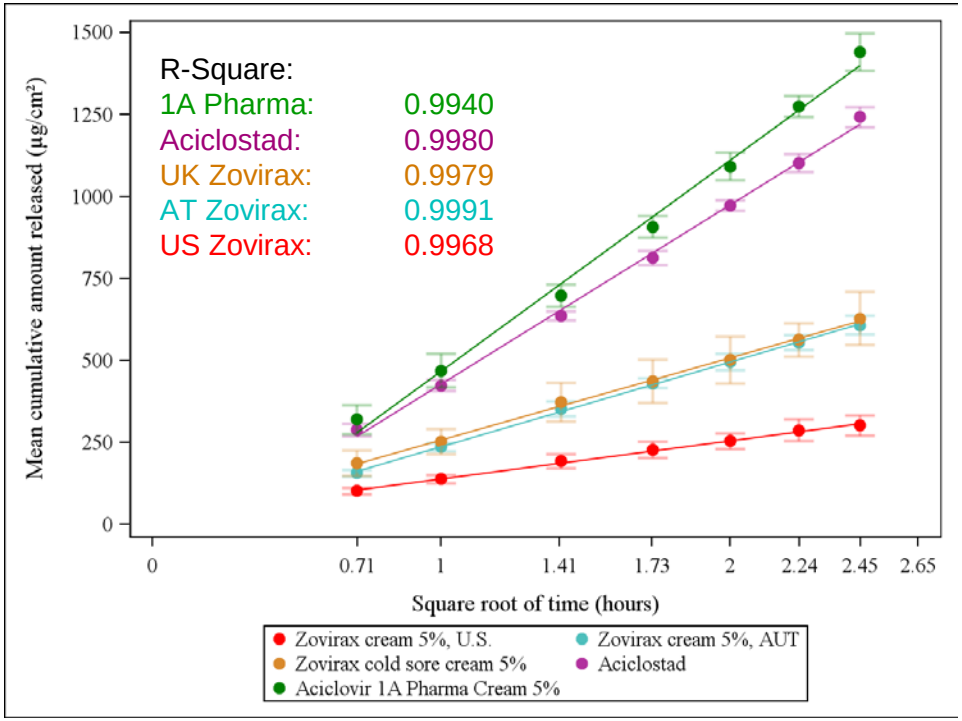
Reference *versus* Zovirax ointment 5% (US)	Non-BE
Reference *versus* Zovirax cream 5% (Austria)	Non-BE
Reference *versus* Zovirax cold sore cream 5% (GSK, UK)	Non-BE
Reference *versus* Aciclostad cream 5% (Austria)	Non-BE
Reference *versus* Aciclovir 1A Pharma cream 5% (Austria)	Non-BE
Reference *versus* Antiviral cold sore cream 5% (Boots, UK)	Non-BE
Reference *versus* Zovirax cream 5% (US)	BE

Methodology

- IVRT study was conducted according to the USP general chapter <1724>
- Pairwise comparison tests **R**eference *versus* **T**est

IVRT
Comparative Study

- ✓ **Non-Zovirax Group (Aciclostad, Aciclovir A1) shows similar behavior**
- ✓ **Zovirax Group (Zovirax AT and UK) shows similar behavior**
- ✓ **Non-Zovirax Group shows higher release rates than Zovirax group**



Bars indicate standard deviation (SD)

In-Vitro In-Vivo Correlation
Summary

In-Vivo

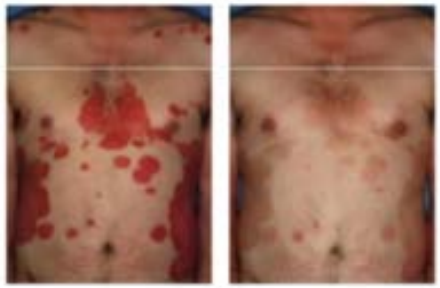
- ✓ dOFM PK profiles of all products are quantifiable for 36 hours
- ✓ Similar rate and extent of bioavailability: Zovirax US vs Zovirax US
- ✓ Different rate and extent of bioavailability: Aciclovir A1vs Zovirax US

IVRT

- ✓ Acyclovir A1 Pharma, Aciclostad > Zovirax UK, AT > Zovirax US
- ✓ Similar release rate : Zovirax US *vs* Zovirax US
- ✓ Different release rate: Zovirax US versus all other products

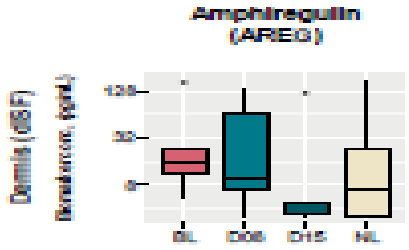
Predictive Bioavailability
The Big Picture

In-Vivo



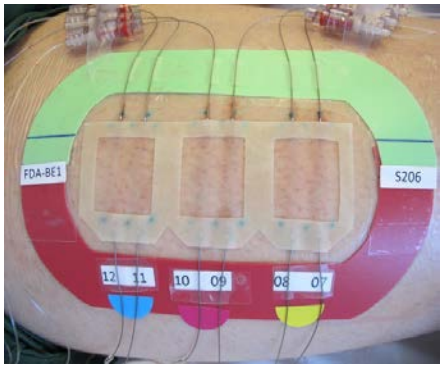
Endpoint Study

In-Vivo

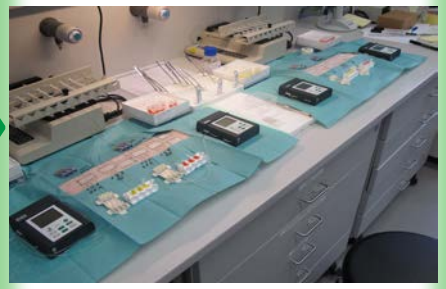


Biomarker Study

In-Vivo



Ex-Vivo



Healthy Human Skin

In-Vitro

e.g. IVRT, IVPT

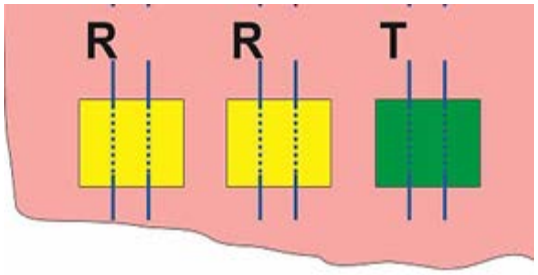
Physico-chem. Prop.

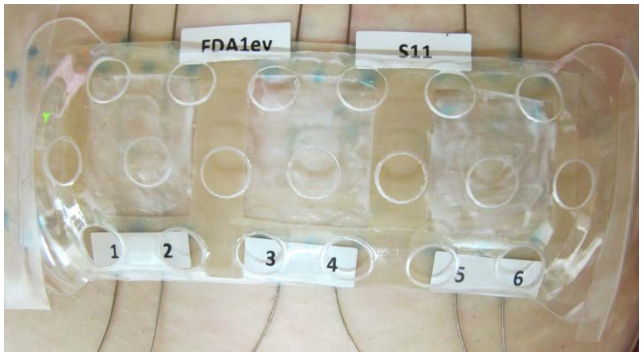
Q1 Q3

Q2

Ex-Vivo
dOFM Study Approach

- Ex-vivo study in excised skin from healthy subjects (n=40)
- *Reference*: Zovirax cream 5% (US)
- *Test*: Aciclovir 1A Pharma Cream 5% (Austria)
- Aims:
  - Investigate BA for *R* vs *R* for 36 h post-dose
  - Investigate BA for *T* vs *R* for 36 h post-dose





dOFM
Conclusion

dOFM

- is highly standardized and reflects the in-vivo skin PK profile
- is able to sample lipophilic and large molecules (up to antibodies) up to 36 hours

dOFM

- showed usability to reflect in-vivo PK differences of topical acyclovir drugs
- proved usability to investigate rate and extent of bioavailability

dOFM may add…

- to In-Vitro In-Vivo Correlation (IVIVC)
- strong support to skin penetration modeling
- the possibility to determine BA in-vivo

….to Pharmacokinetics-Based BA Approaches

A big Thanks to…

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Clinical PI

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BE Expert

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FDA Project Officer

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Data Mangaement

Thomas Augsutin
Statistics

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Jill Coker

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Thank you for your attention

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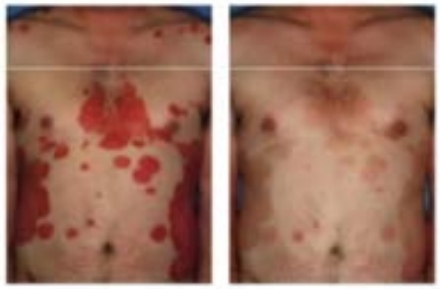
dOFM

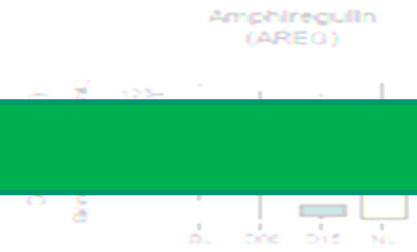
There is a Method Available to Assess In-Vivo PK in Dermis

In-Vivo



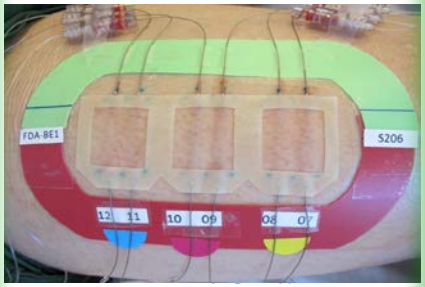
Endpoint Study

In-Vivo



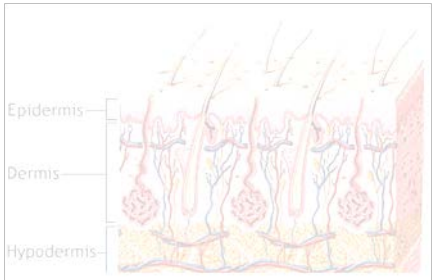
Biomarker Study

In-Vivo



PK Healthy Subjects

Ex-Vivo



Healthy Human Skin

In-Vitro

e.g. IVRT, IVPT

Physico-chem. Prop.

Q1 Q3

Q2