



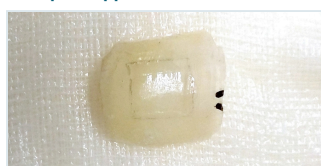
Why infected nails matter. In our published series, mycosis-infected toenails measured 0.74–4.04 mm thick (mean 2.38 ± 1.01 mm, $n = 18$) against 0.72 ± 0.20 mm for healthy nails ($n = 14$) — more than three times thicker. A penetration result obtained on healthy nails does not transfer to the diseased nail a patient actually has.

NAIL PREPARATION AND DOSING — RUN AT PKDERM

Nail material	Ex vivo human cadaver toenails (big toe), ethically sourced under the regulations applicable in France. Healthy nails, or mycosis-infected nails when the diseased matrix is the question. Stored individually at -20°C .
Preparation	Thawed at room temperature, immersed in 70% ethanol for 15 minutes, washed twice in deionised water. Thickness measured on at least three zones per nail with an electronic external gauge, and the mean reported.
Filing	Where the approved posology of the product requires it, nails are filed with an emery board (four forward and four backward motions). Filed and intact nails can be compared directly — in one published series, filing increased amorolfine recovered in the nail plate by around 40-fold.
Application	A 1 cm^2 zone is marked; the nail rests on gauze moistened with sterile water. The formulation is applied at a defined dose per cm^2 , following the product's real treatment procedure, then air-dried.
Remanence	Humidified incubator at controlled temperature for the remanence period under study — typically 6 or 24 hours after a single application, or a repeated-dose schedule.
Decontamination	The nail surface is cleaned with acetone-wetted swabs before sectioning, so that residual surface product is not mistaken for penetrated drug. This step decides whether the result means anything.
Cryo-sectioning	The treated area is embedded in carboxymethylcellulose gel with the growth axis parallel to the stage, frozen, and cut on a cryo-microtome at -21°C . Sections of 10 or 20 μm along the growth axis, taken consecutively from the surface down.

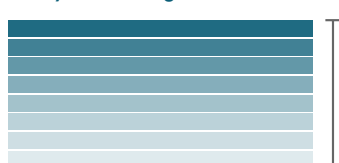
HOW THE LAYERWISE PROFILE IS BUILT — the same sections feed analytical quantification or MALDI-MSI

1 · Topical application



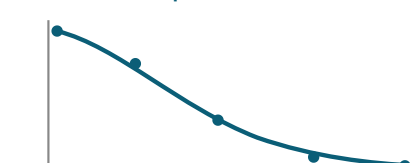
1 cm^2 zone marked on the nail, defined dose, 6 or 24 h remanence, then surface decontamination.

2 · Cryo-sectioning



cut along the growth axis, from the dorsal surface down

3 · Concentration profile



amount recovered per layer, against depth

READ-OUTS

Spatial distribution by MALDI-MSI

Mass spectrometry imaging of the drug substance across the nail section, in positive ion mode at 20–30 μm pixel size, showing where the molecule actually sits in the nail plate. In our published work this distinguished a compound diffusing homogeneously through the full nail plate within 6 hours from compounds that stayed confined to the uppermost layer. Imaging is performed by our long-standing partner, the **University of Copenhagen**.

Layerwise quantification

Consecutive sections are solubilised and the test item quantified layer by layer, giving a concentration profile through the nail plate rather than a single end-point value. Analytical quantification is carried out at qualified partner laboratories, as for our skin permeation work.

Antifungal efficacy

The mycology bioassay — agar diffusion against *Trichophyton rubrum* with a concentration–zone-of-inhibition reference curve — is not run in our laboratory. We prepare, treat and section the nails, and work with a specialist mycology laboratory, ours or yours.

TYPICAL QUESTIONS

Comparative penetration	Your formulation against one or more marketed references, on nails from the same donor set and under each product's own approved application procedure.
Formulation development	Vehicle, film former and penetration enhancer compared on the same nails.
Product interaction	Whether an overlying product affects a medicated lacquer — in one published series, eleven best-selling cosmetic nail varnishes were applied over an antifungal lacquer and the subungual antifungal activity was read on human nails.
Claim substantiation	There is no validated OECD guideline for nail permeation. Protocols are built around the claim, with acceptance criteria agreed in writing before the start.
Related capability	The same laboratory runs in vitro permeation testing on human skin under OECD TG 428, so a topical product can be characterised on skin and on nail by one provider, to one reporting standard.
What we do not do	We do not run the mycology bioassay in house, and we say so up front. Everything upstream of it — sourcing, characterisation, dosing under the real posology, decontamination and sectioning — is where the result is won or lost, and that is what we do.

PUBLISHED WORK

- Endringer Pinto F, Bagger C, Kunze G, Joly-Tonetti N, Thénot J-P, **Osman-Ponchet H**, Janfelt C. Visualisation of penetration of topical antifungal drug substances through mycosis-infected nails by matrix-assisted laser desorption ionisation mass spectrometry imaging. *Mycoses* 2020;63:775–781. Nail treatment and cryo-sectioning performed at PKDERM; MALDI-MSI at the University of Copenhagen.
- Ghannoum M., Long L., Kunze G., Sarkany M., **Osman-Ponchet H**. A pilot, layerwise, ex vivo evaluation of the antifungal efficacy of amorolfine 5% nail lacquer vs other topical antifungal nail formulations in healthy toenails. *Mycoses* 2019;62:494–501.
- Sigurgeirsson B., Ghannoum M.A., **Osman-Ponchet H**, Kerrouche N., Sidou F. Application of cosmetic nail varnish does not affect the antifungal efficacy of amorolfine 5% nail lacquer in the treatment of distal subungual toenail onychomycosis: results of a randomised active-controlled study and in vitro assays. *Mycoses* 2016;59:319–326.

WHAT YOU RECEIVE

Study protocol

Agreed before start, with acceptance criteria stated up front.

Raw and processed data

Full data set, calculations and quality-control records.

Study report

Interpretation, figures and conclusions for your file.

Scientific support

Discussion of results and advice on the next study.