

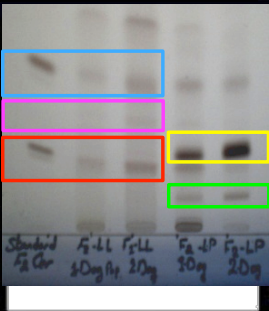
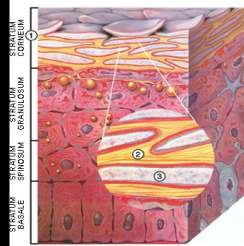
## Using HPTLC as a non-invasive method for the study of skin barrier function

Utilisation de l'HPTLC comme méthode non-invasive dans l'étude de la fonction barrière de la peau

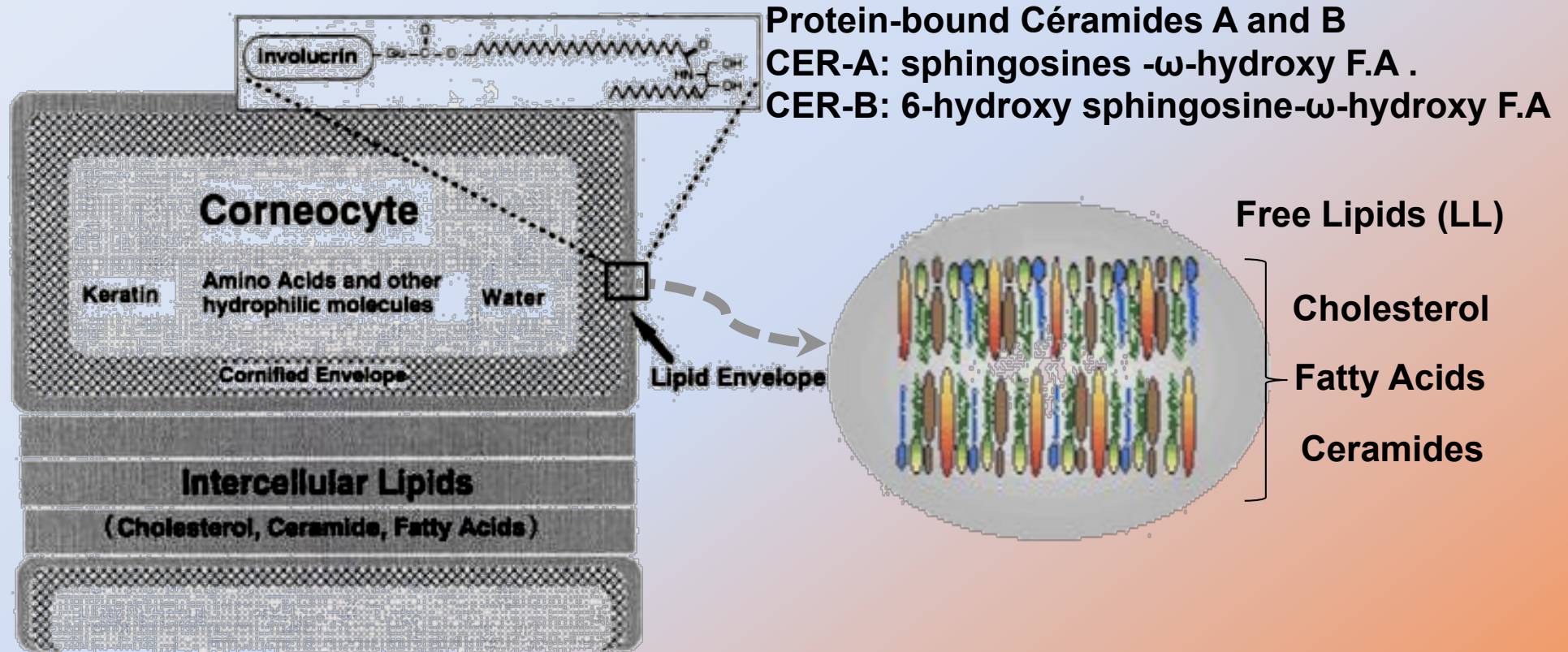
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# Intercellular Lipids:



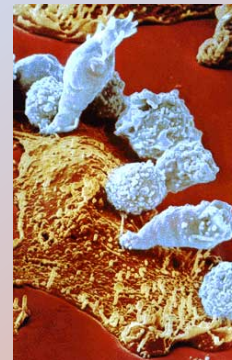
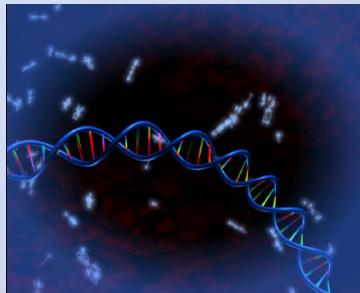
# What Is Atopic Dermatitis ?



## Atopic dermatitis (AD):

- Is a common, chronically relapsing inflammatory skin disease of unknown etiology with an impaired permeability barrier function.
- Affects especially children, as well as young adult.
- Characterized by complex interaction between genetic & environmental factor:

- Life style
- Family size
- Urban or rural areas
- Age of the mother
- Breastfeeding



AD affects up to 20% of children in Europe.  
(Keratin 2005; 9:24-29/ J.MAZEREEW)

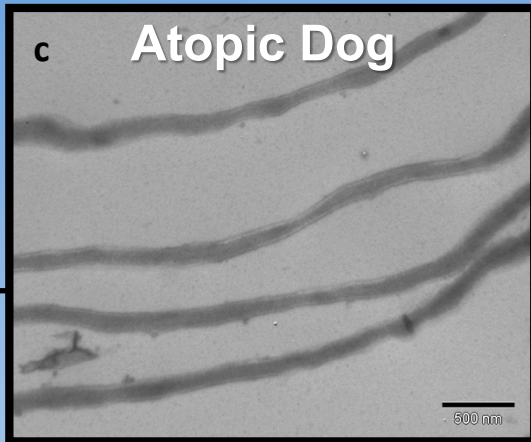
## Why DOG model for Atopic dermatitis study:

- Dog could be a suitable model for studies on physiopathology prevention and treatment of human AD
- Mammalian species.
- Similarity between canine and human AD, both are characterized by an impaired epidermal barrier.
- Less obstacles ( collection of samples, applying medication, privacy...)

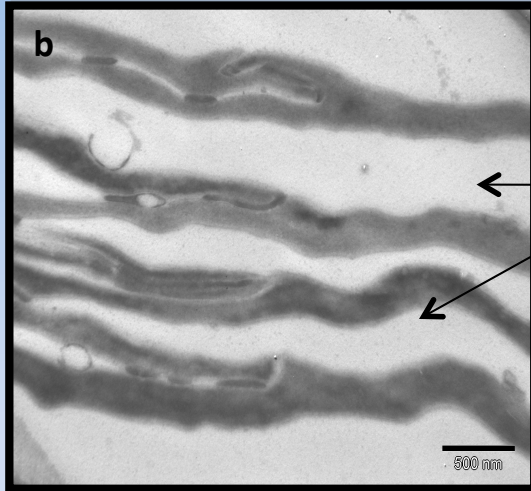


**Transmission Electron**  
**Microscopy**  
**results with Dogs Epidermis**

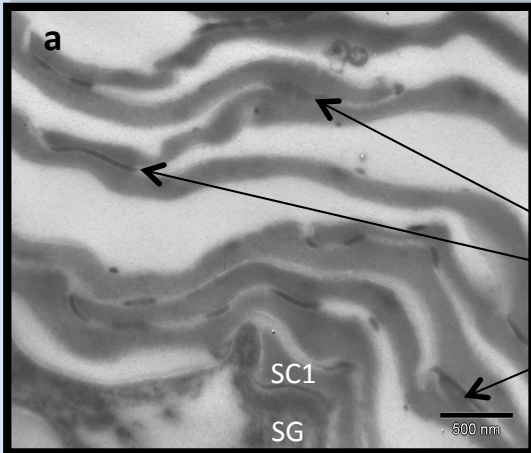
Superior-SC



Intermediate-SC



Inferior-SC



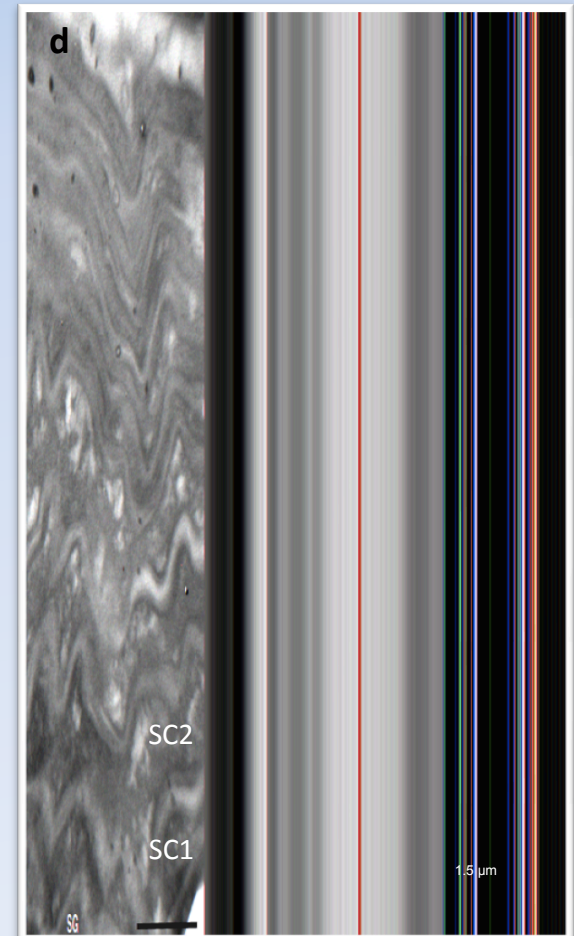
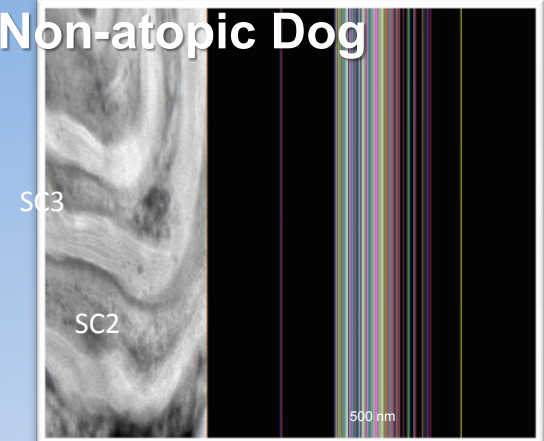
Intercorneocyte  
space filled by  
Lamellar lipid

Intercorneocyte  
space empty

Corneodesmosome

SC: Stratum Corneum; SG: Stratum Granulosum

e Non-atopic Dog



Investigation of atopic dermatitis (AD) and non-atopic canine epidermal lipids modification of expression, and organization by means of:

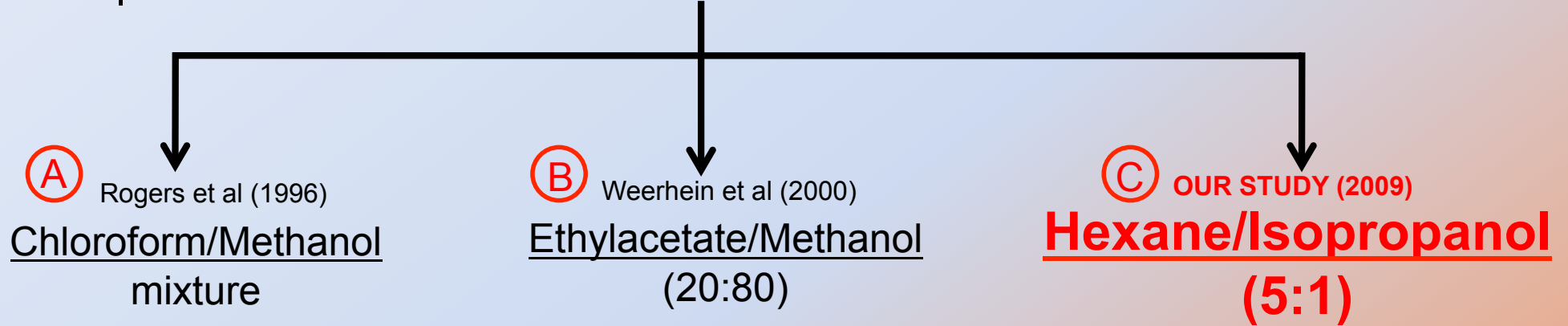
- Tape Stripping.
- High Performance Thin-Layer Chromatography (HPTLC).



# Methods for Skin Lipids Extraction:

1. Collection of upper Stratum Corneum (SC) by several consecutive tape strips

Extraction with



2. Collection of six consecutive scrappings followed by extraction with chloroform/methanol (*Lavrijsen et al. 1995*)

3. Collection of SC by stripping with cyanoacrylate (*Bleck et al. 1999*)

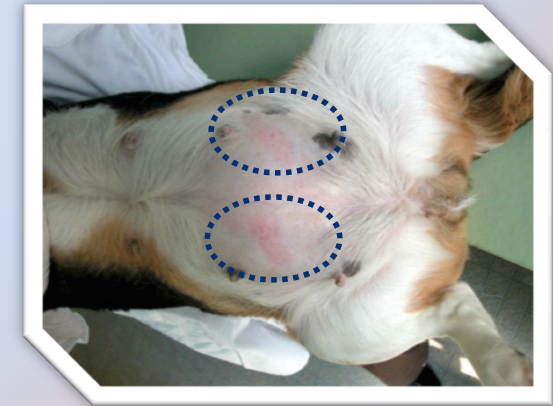
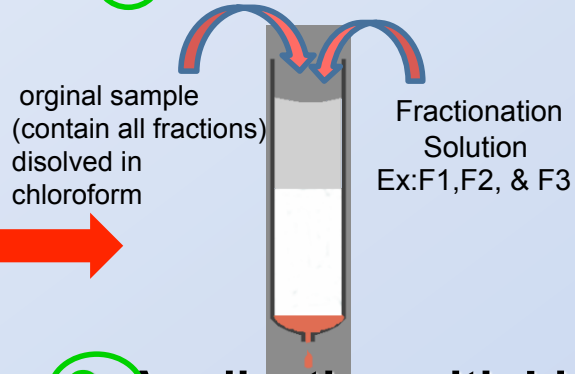


# Tape stripping in combination with HPTLC to assess SC lipid:

## (A) Extraction



## (B) Fractionation



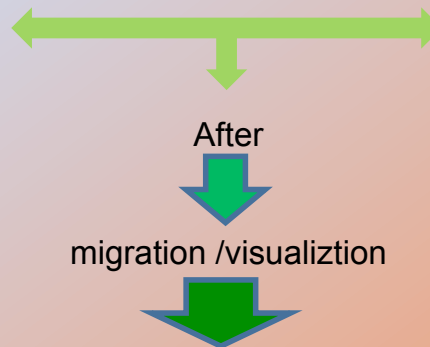
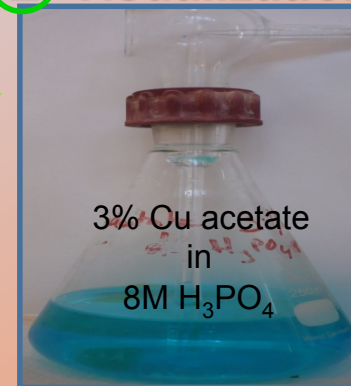
## (C) Application with Linomat IV



## (D) Migration

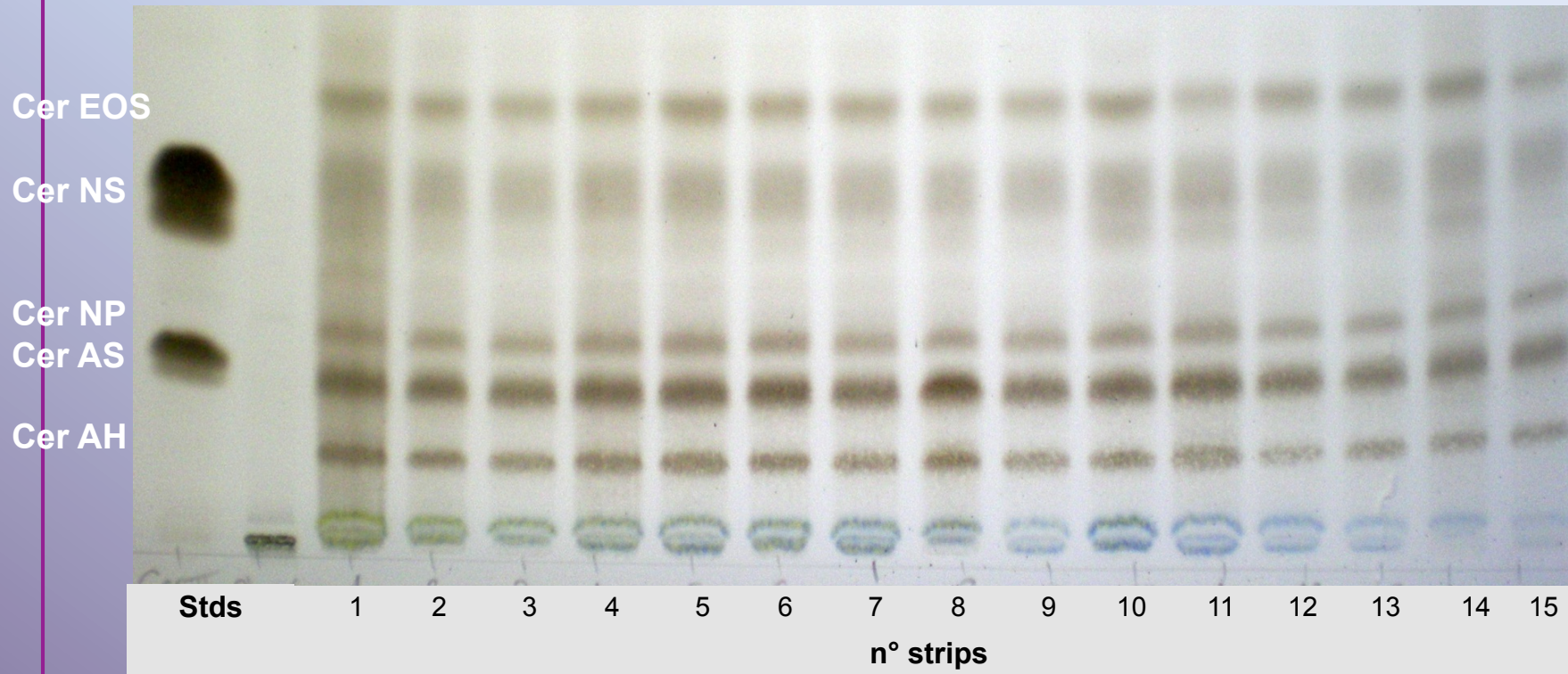


## (E) Visualization





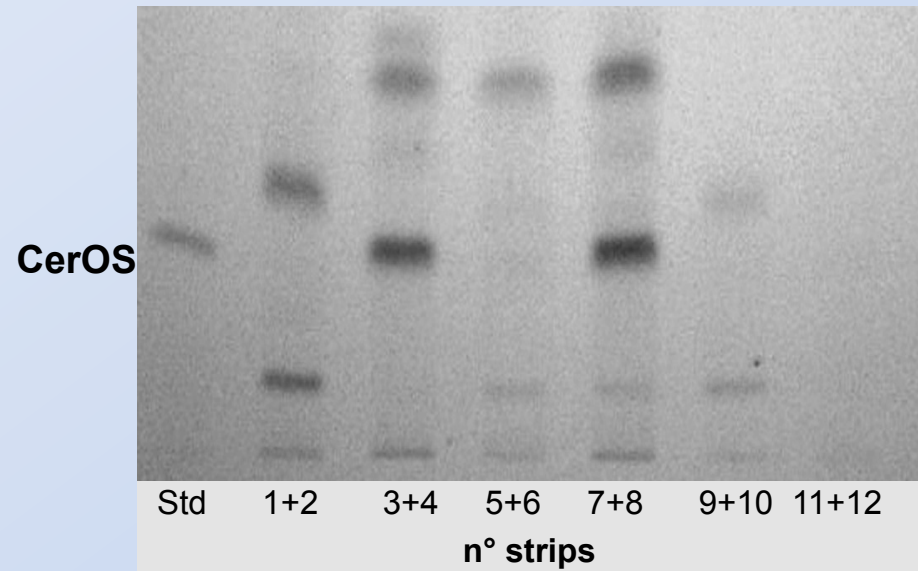
## Free ceramides of healthy human Stratum Corneum



### Conclusion:

The homogeneity of the stratum corneum is observed on healthy human skin  
The uptake of consecutive strips shows a homogeneous distribution consistent with an efficient barrier function .

➤ **Atopic human stratum corneum (strips pooled 2 by 2)**

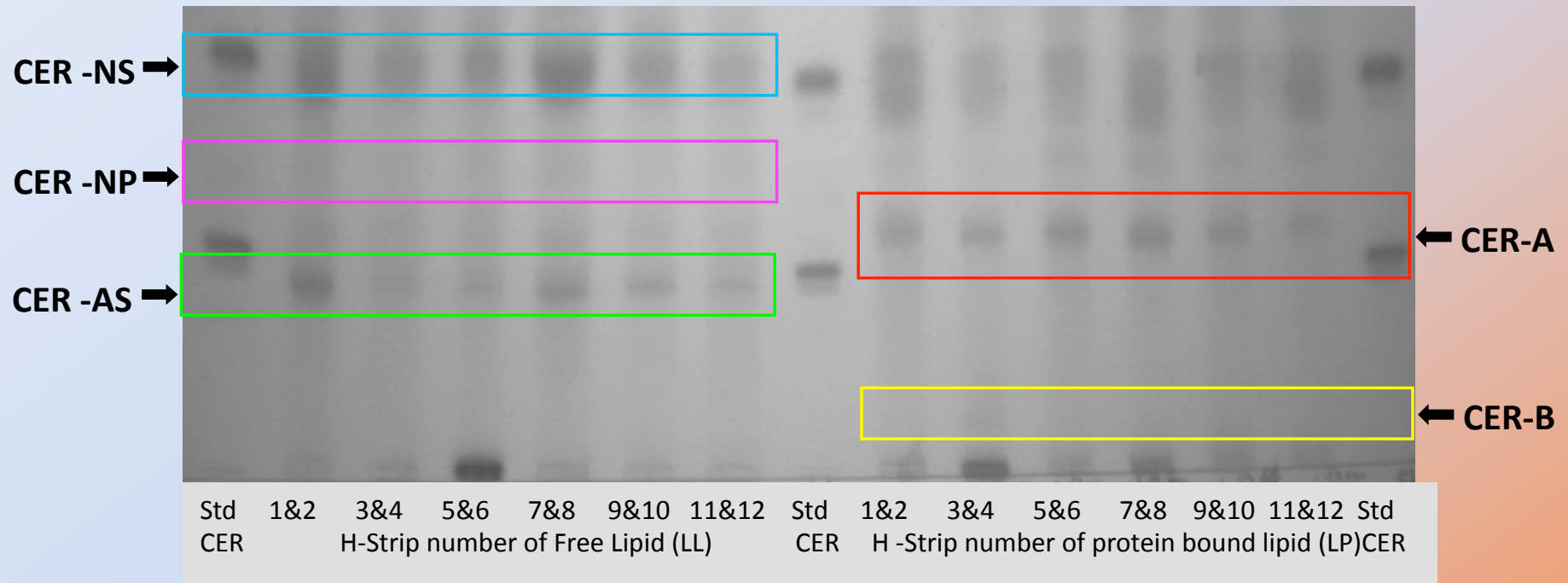


**Conclusion:**

**Heterogeneity of the lipid content in these strips suggesting a disorganized Structure of Stratum Corneum leading to a altered barrier function !**

## ➤ Healthy Dog (2 by 2 strips)

### Free Ceramides and Protein-bound ceramides



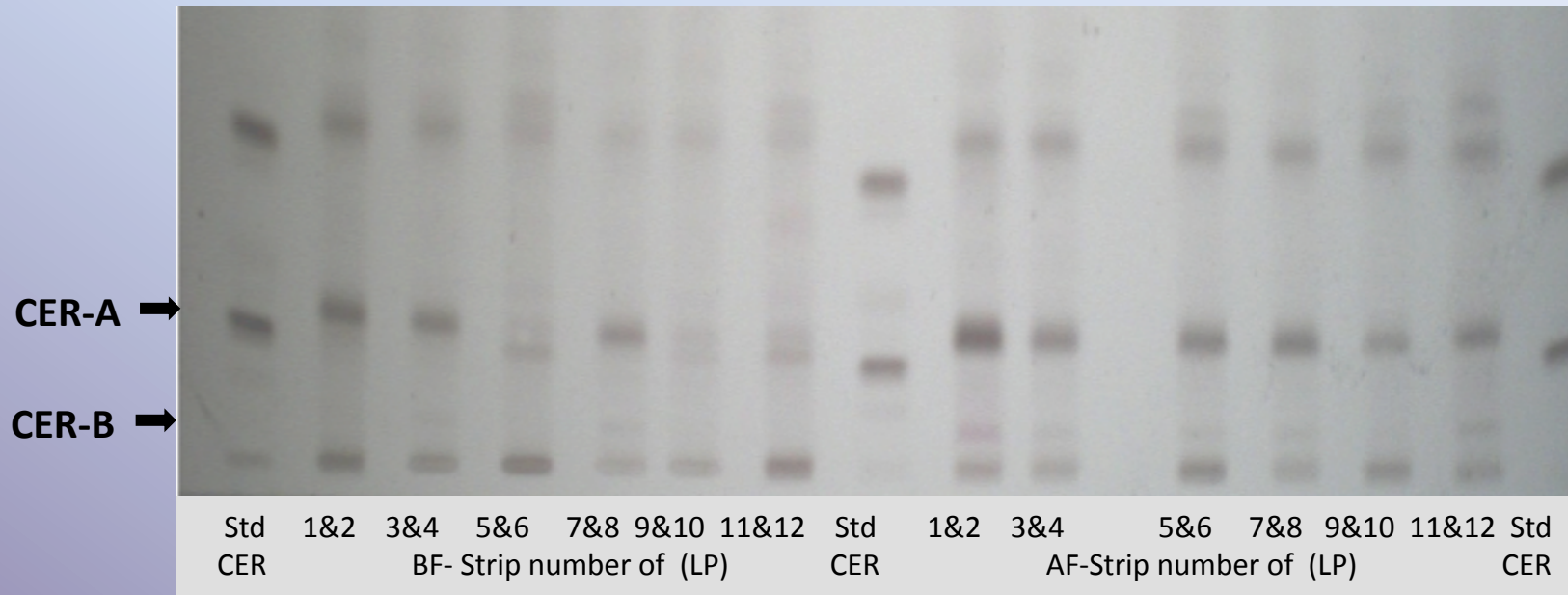
Std-CER: Standard Ceramide ;  
 CER-NS: Ceramide2 (normalFA- sphingosine);  
 CER-NP: Ceramides 3 (normalFA- phytosphingosine);  
 CER-AS: Ceramide5 ( $\alpha$  hydroxyFA- sphingosine);

CER-A: covalently bound ceramides Cer (OS)  
 sphingosine- $\omega$ -hydroxy F.A;  
 CER-B: covalently bound ceramides Cer (OH)  
 6hydroxy sphingosine- $\omega$ -hydroxy F.A.

**Conclusion: heterogeneous distribution of ceramides in the strips**

## ➤ Atopic Dog ( Strips as 2 by 2)

### Protein bound Ceramides before and after treatment



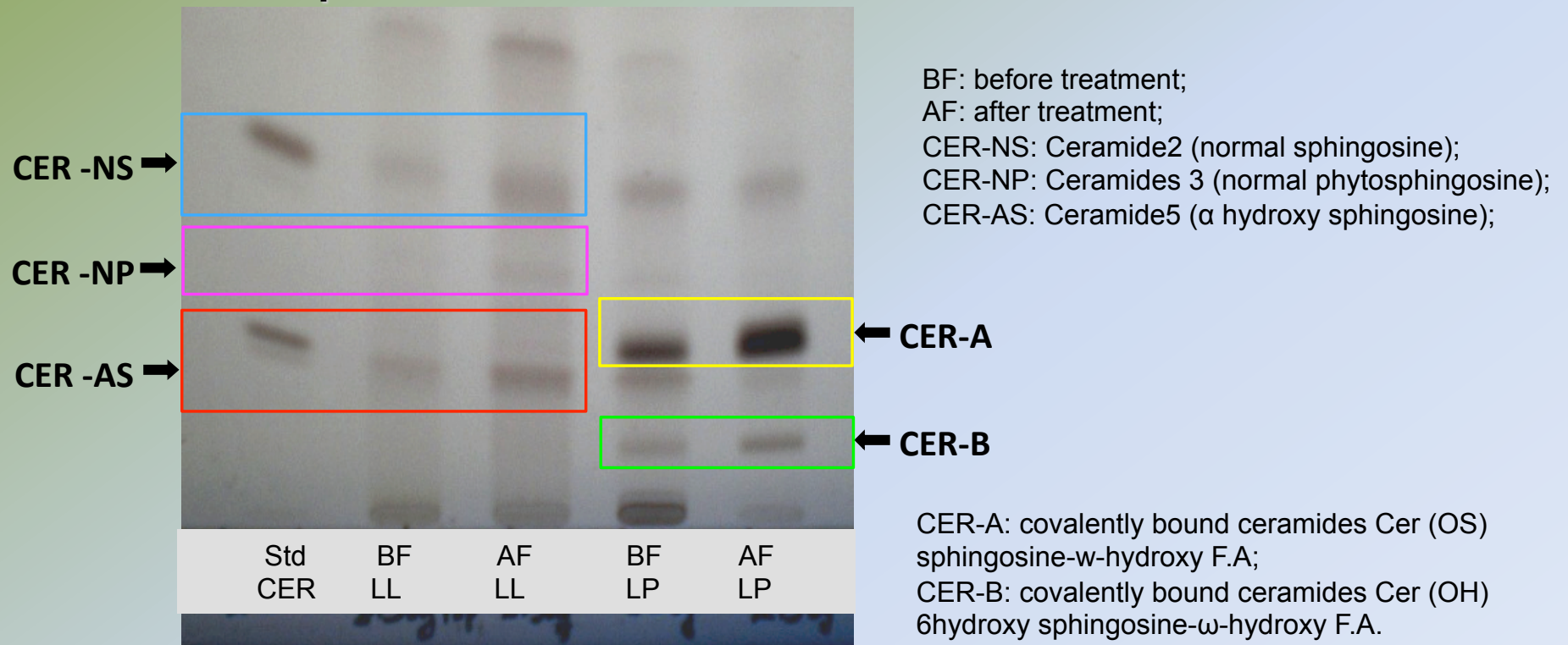
Std-CER: Standard Ceramide (0.25mg/ml); BF: before treatment; AF: after treatment;  
CER-A: covalently bound ceramides Cer (OS) sphingosine- $\omega$ -hydroxy F.A;  
CER-B: covalently bound ceramides Cer (OH) 6hydroxy sphingosine- $\omega$ -hydroxy F.A.

### Conclusion:

- Homogenous elevation in bands expression after treatment with Megaderm®

## ➤ Atopic Dog ( Strips as Pool )

### Free and protein bound Ceramides before and after treatment



### Conclusion:

The strips taken as a pool give an information on the lipid content of the Stratum Corneum but without any information on the distribution in different layers.

In this case it gives information on the increase in lipid content after topical treatment with Megaderm®

## Conclusion:

- Studies on the lipid content of the Stratum Corneum using the tape stripping method coupled with HPTLC give quantitative and qualitative informations about the distribution of the different lipids in consecutive layers.
- Such a detailed information cannot be obtained by electron microscopy which does not discriminate between the consecutive layers.
- This method has the advantage of being non-invasive, which is important when it must be applied on children as it is mostly the case with human atopic dermatitis.

