

UNISART lateral flow membranes - consistency by design

Standard hCG test performance

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Outline

- Introduction to lateral flow immuno assays
- Description of the standard hCG test @ Sartorius AG
- Factors that influence standard hCG test performance
 - test architecture
 - drying conditions
 - running conditions
- Different methods to detect capture antibody deposition
 - gold particle detection by vapor pressure SEM
 - direct detection by fluorescent microscopy

Introduction to immunochromatographic assays

=> membrane based assays

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Objective of immunochromatographic assays:

=> fast, qualitative or quantitative, analysis of biological or chemical entities without special tools or sample preparation

Characteristics:

=> based on antibody-antigen (immuno) reactions

=> readable signal response (color, fluorescence, magnetic)

=> sensitive: down to 10^{-12} grams/ml

Membrane based formats:

=> lateral flow

=> flow through

Applications:

=> detection of diseases, physical conditions, drugs of abuse in body liquids

=> detection of biological or chemical entities in environment or food

Why cellulose nitrate membranes?

structural properties:

=> high surface per volume ratio: approx. $30-100 \text{ cm}^2/\text{cm}^3$

=> capillary properties: 3-8 μm pores with open cell structure

=> thin: down to 50 μm

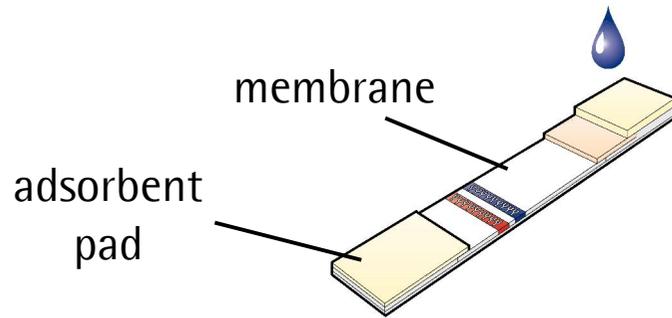
material properties:

=> high non-specific binding of proteins with minimal denaturation

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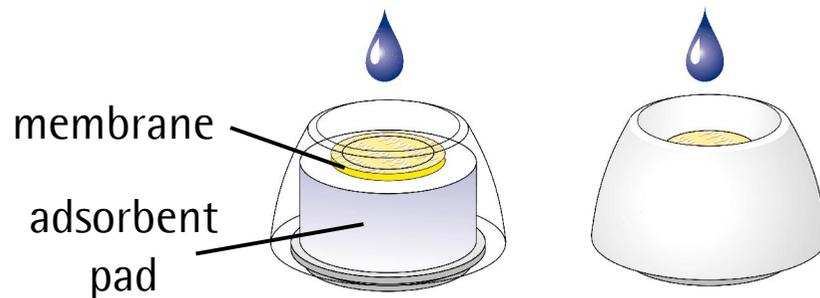
Membrane based rapid-test formats:

=> lateral flow



Cellulose nitrate membrane with large pore size: $5\mu\text{m}$ to $15\mu\text{m}$ to enable rapid capillary flow

=> flow through



Cellulose nitrate membrane with smaller pore size: $0.2\mu\text{m}$ to $3\mu\text{m}$

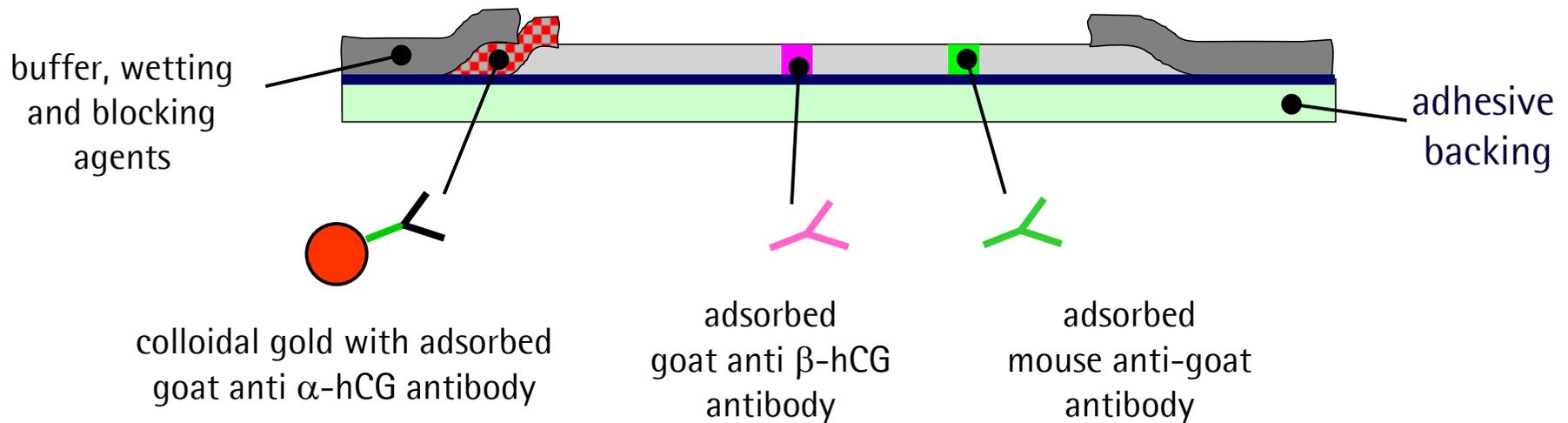
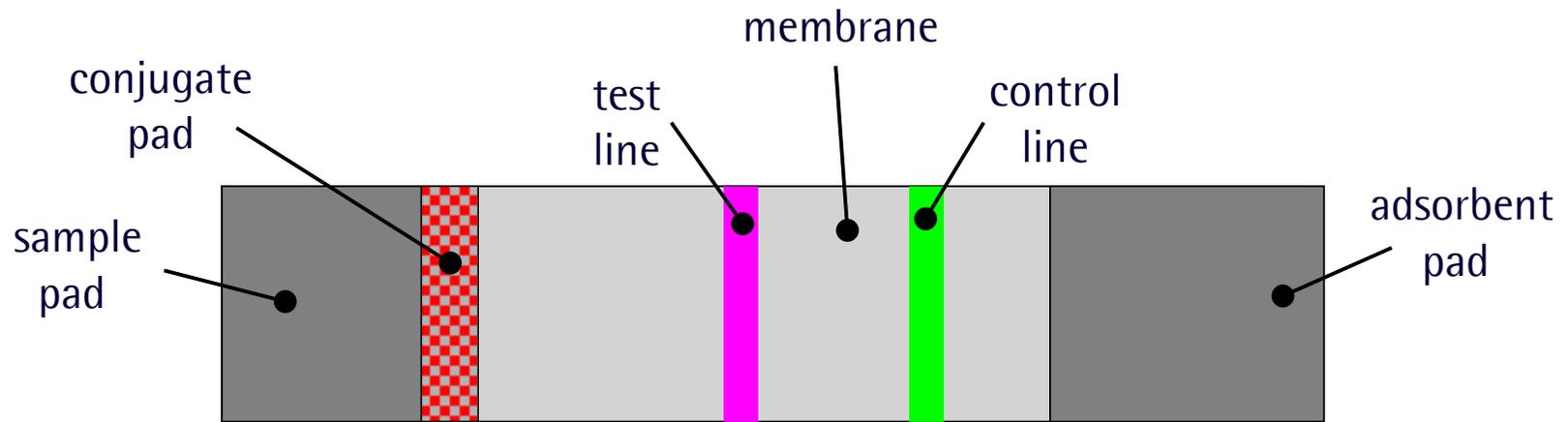
Typical applications of lateral flow immuno-chromatographic tests:

- Fertility
 - Infectious disease
 - Drugs of Abuse
 - Tumor markers
 - Cardiac markers
- and others.....

Typical markers for analyte detection

- colloidal gold
- PS-latex beads
- magnetic beads
- dyes

UNISART lateral flow graph in tests hCG consistency by design

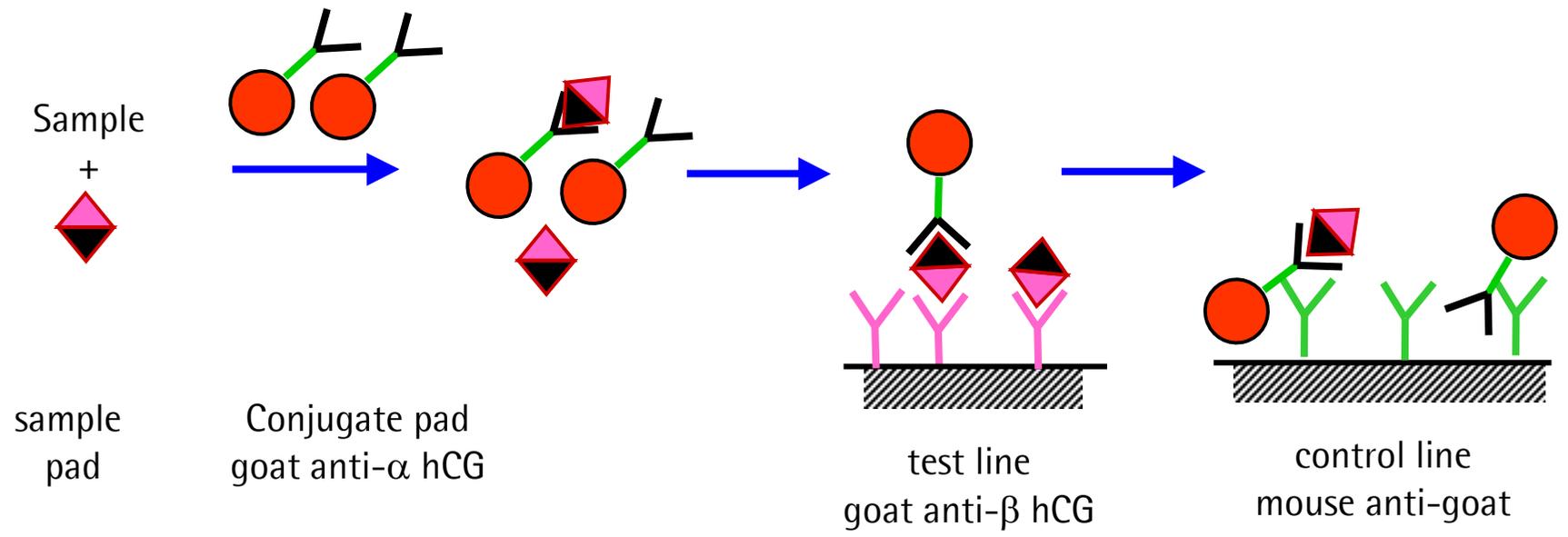
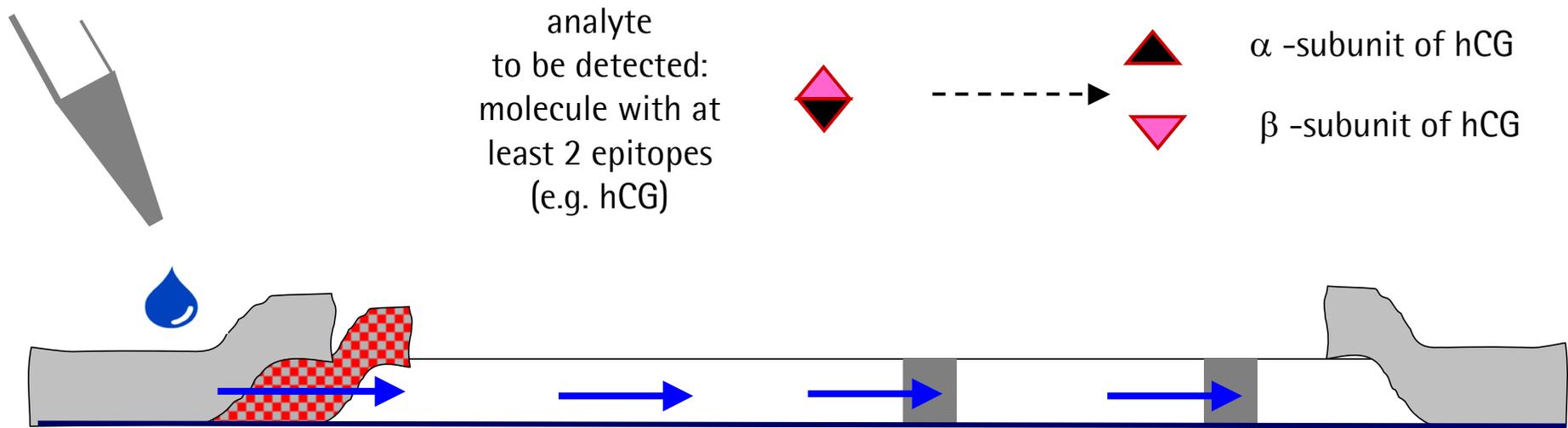


*hCG = humane chorion-gonatotropin

CONFIDENTIAL

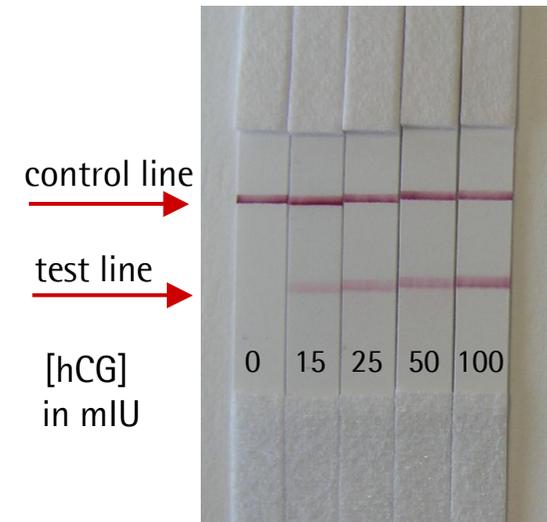
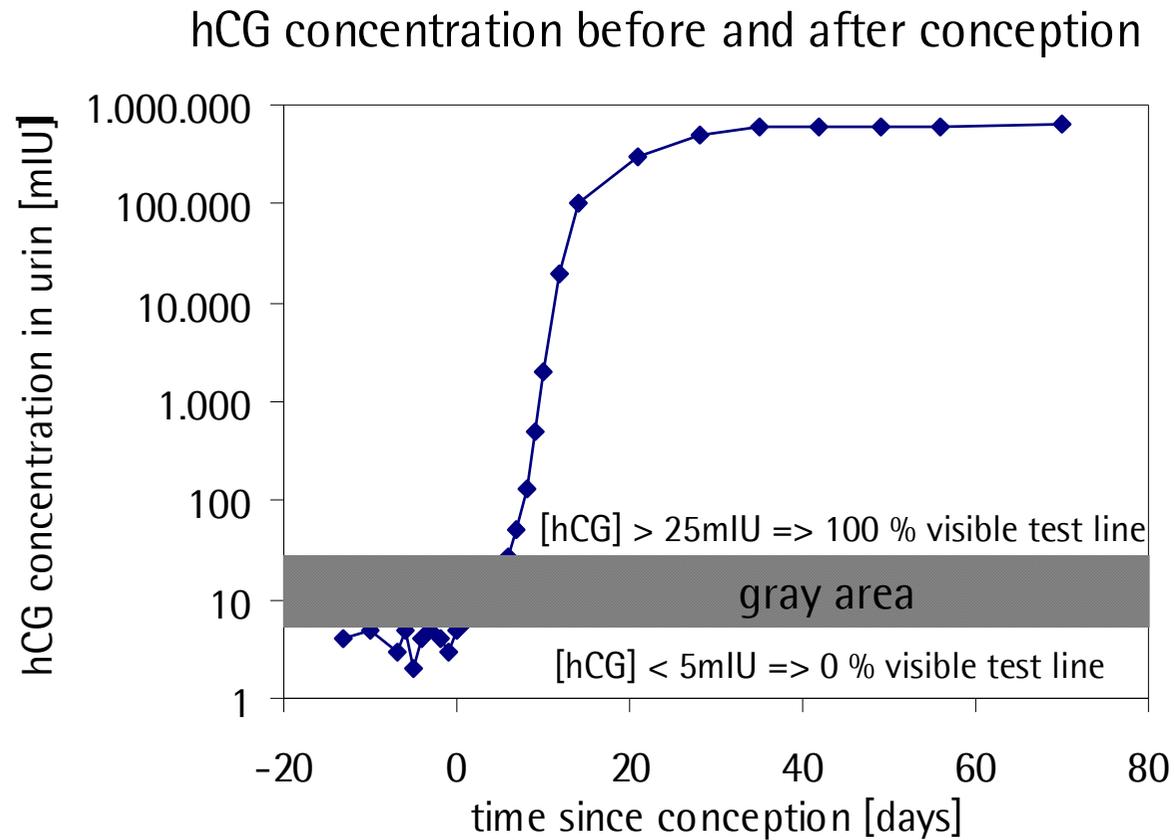


UNISAPITe lateral flow test strips hCG in saliva by design



=> result: test line is colored if analyte is present

UNISART lateral flow graph in test set - Consistency by design



Description of standard hCG test @ Sartorius AG

Used for:

=> benchmarking

=> membrane development

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Standard hCG test: components

Sample pad

None (not necessary as we use buffered analyte)

Conjugate pad

Glassfibre, Reemay, type: 2040, width: 2,5 cm

Adsorbent pad

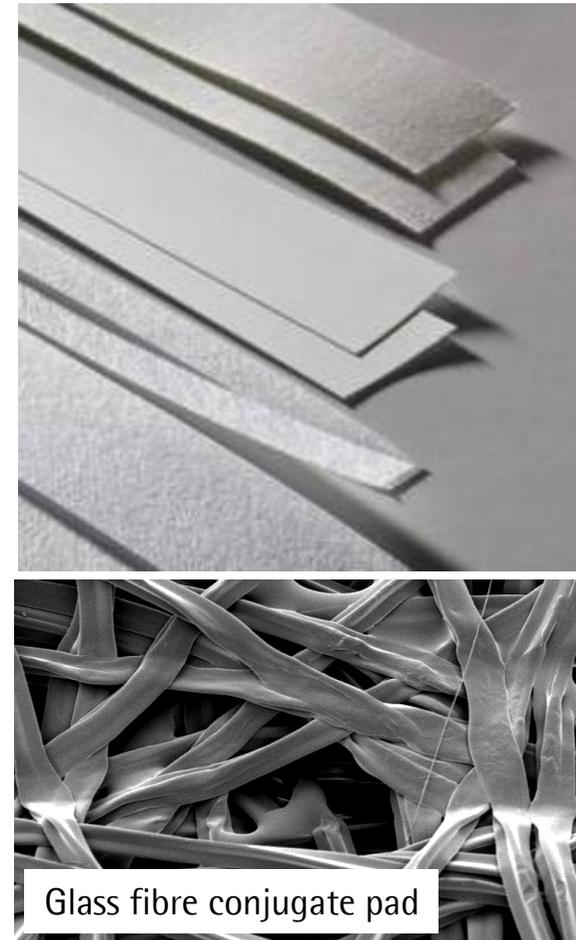
Cellulose, Whatmann, type: 17 Chr, width: 2,5 cm

Plastic backing

G&L Precision Die Cutting Inc., size: 7,5 x 34,7 cm

Membrane

Different materials



Standard hCG test: impregnation/blocking

Buffer for conjugate pad
impregnation

100 mM	Tris, pH 8
0,5% (w/v)	BSA
0,25% (w/v)	Tween20

=> pad is soaked in impregnation buffer and left to dry at room temperature over night



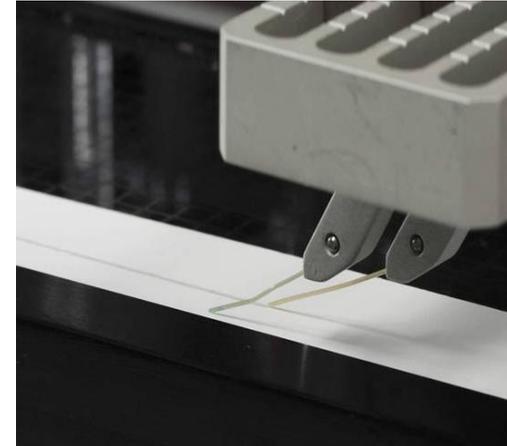
Standard hCG test: components

Test line

Antibody: Anti- α -hCG from goat (Arista Biologicals, USA)
Concentration: 4 mg·ml⁻¹
Buffer: 5 mM borat w. 1% (w/v) Saccharose, @ pH 8
Striping: 1 μ l·cm⁻¹
Striping speed: 50 mm·s⁻¹

Control line

Antibody: Rabbit Anti-mouse IgG (Alchemy, GB)
Concentration: 1 mg·ml⁻¹
Buffer: see above
Striping: see above
Striping speed: see above



=> Test- and control-line are printed with a contact system

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Standard hCG test: components

Gold conjugate

Particles: 40 nm gold particles (Alchemy, GB)
Antibody: Anti- β -hCG from goat



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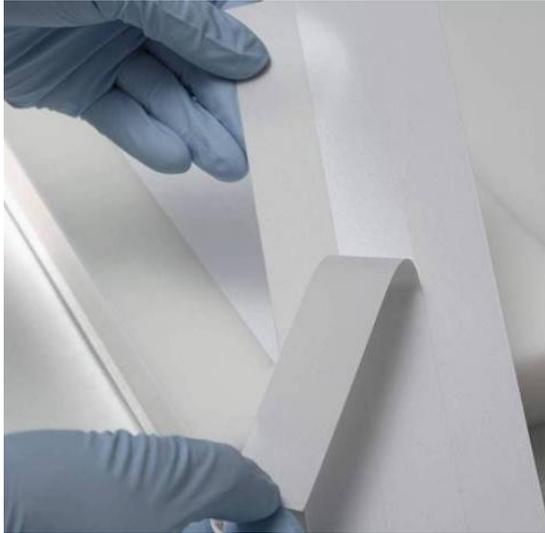
Standard hCG test:
Test- and control-line drying

=> 30 min at 60°C

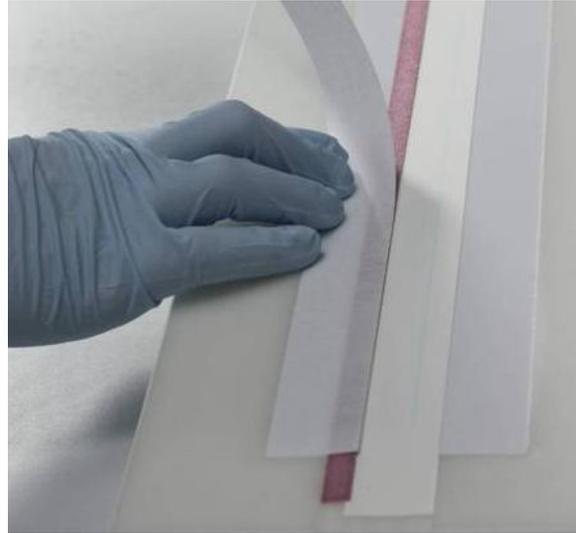


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Standard hCG test: assembly



Prepare backing



Laminate
components

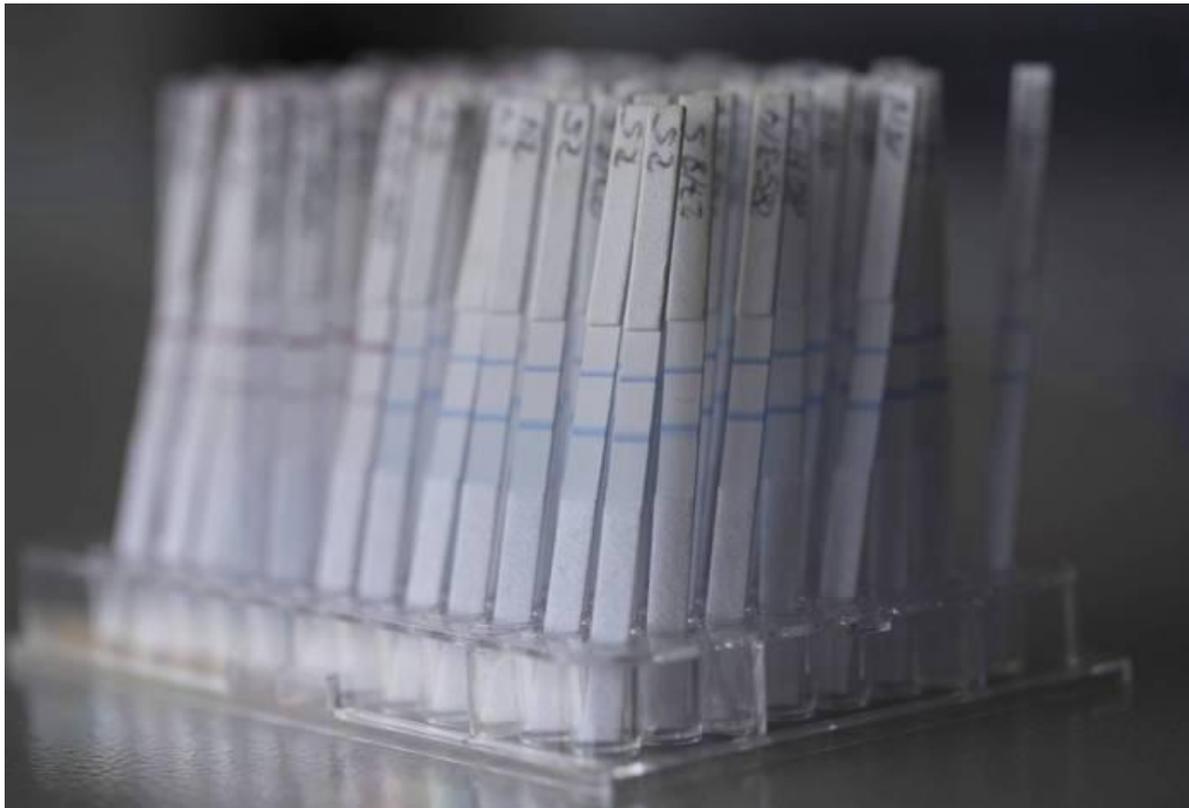


Apply pressure
reproducibly

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Standard hCG test: running the test

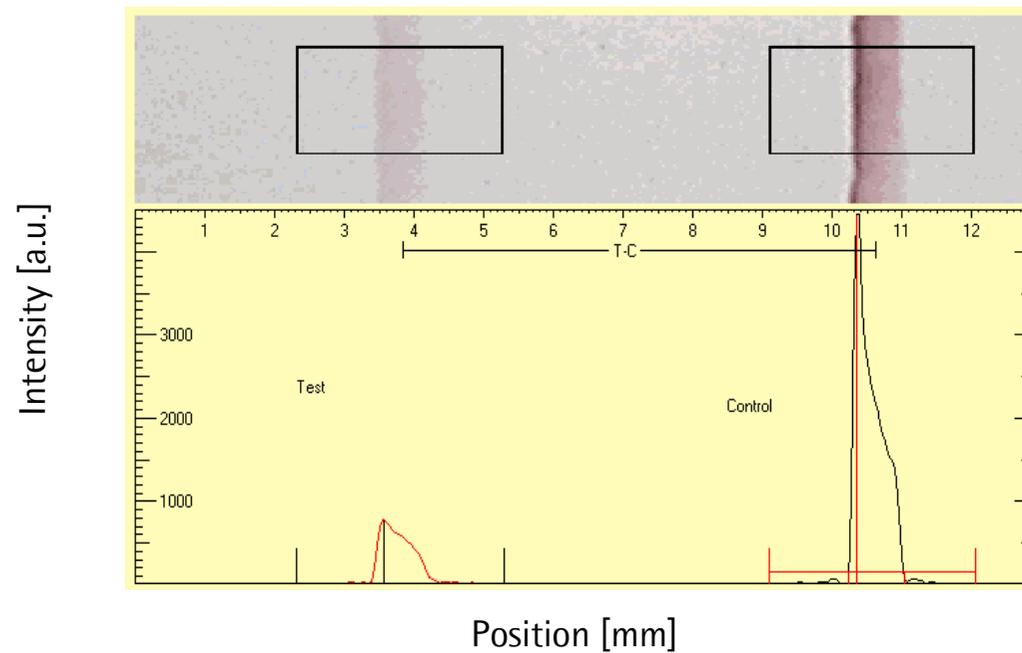
=> run test in 140 µl analyte buffer in well plate



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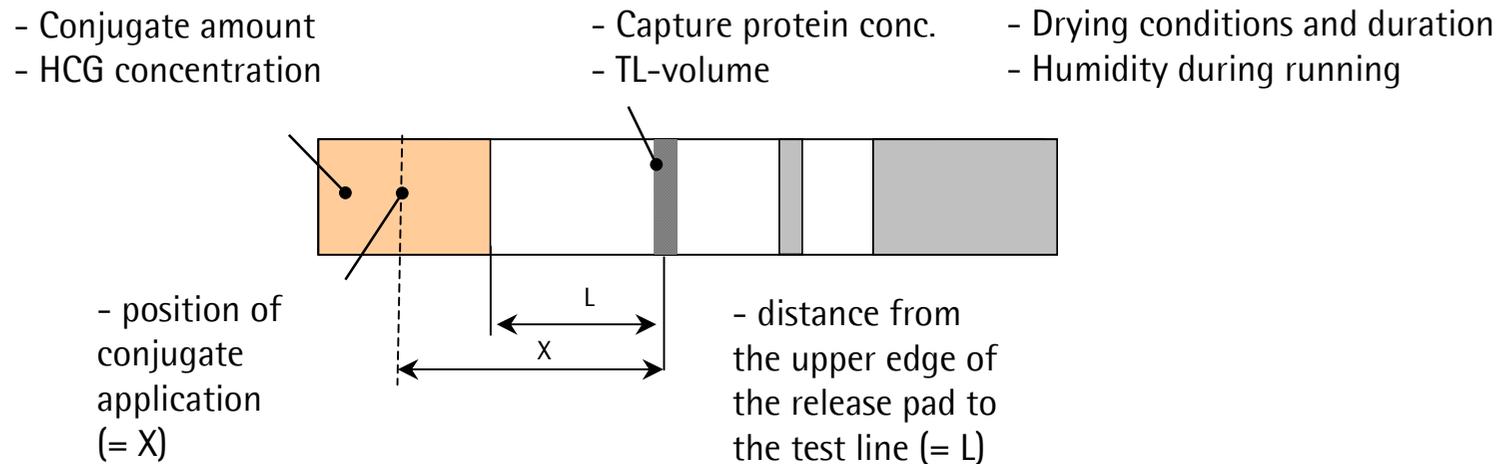
Standard hCG test:

=> Evaluate line intensity with reader



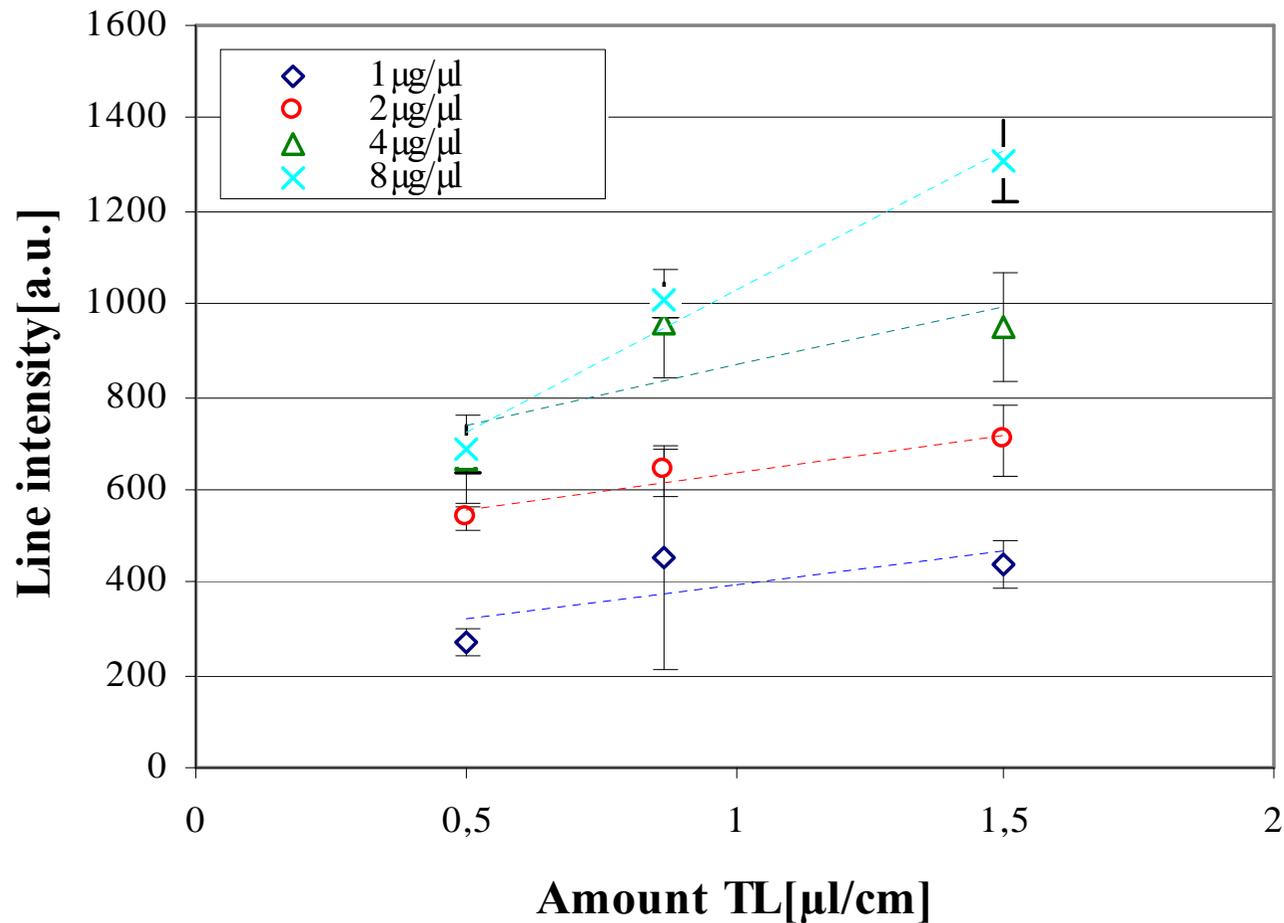
Influencing factors

Illustration of varied factors in the hCG Test



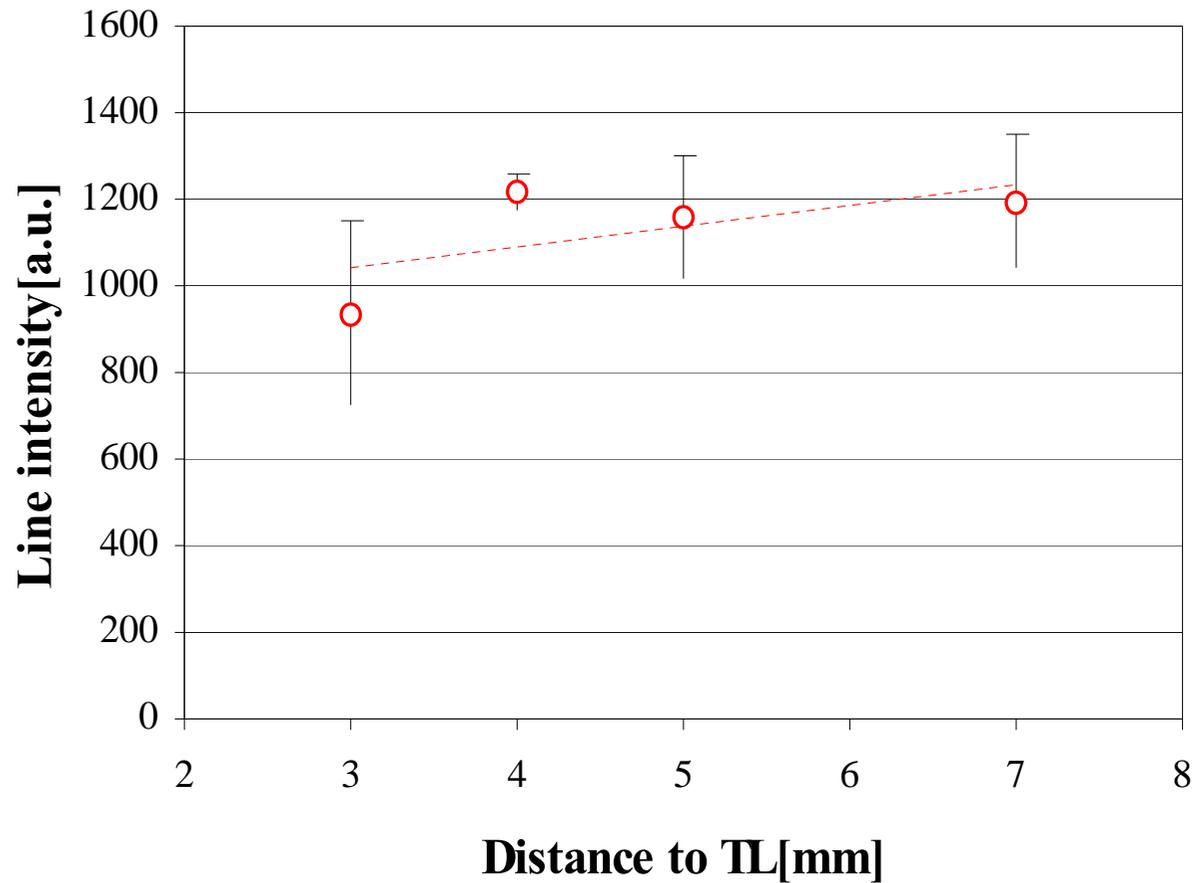
Influencing factors
=> test architecture

Test line concentration and printed volume



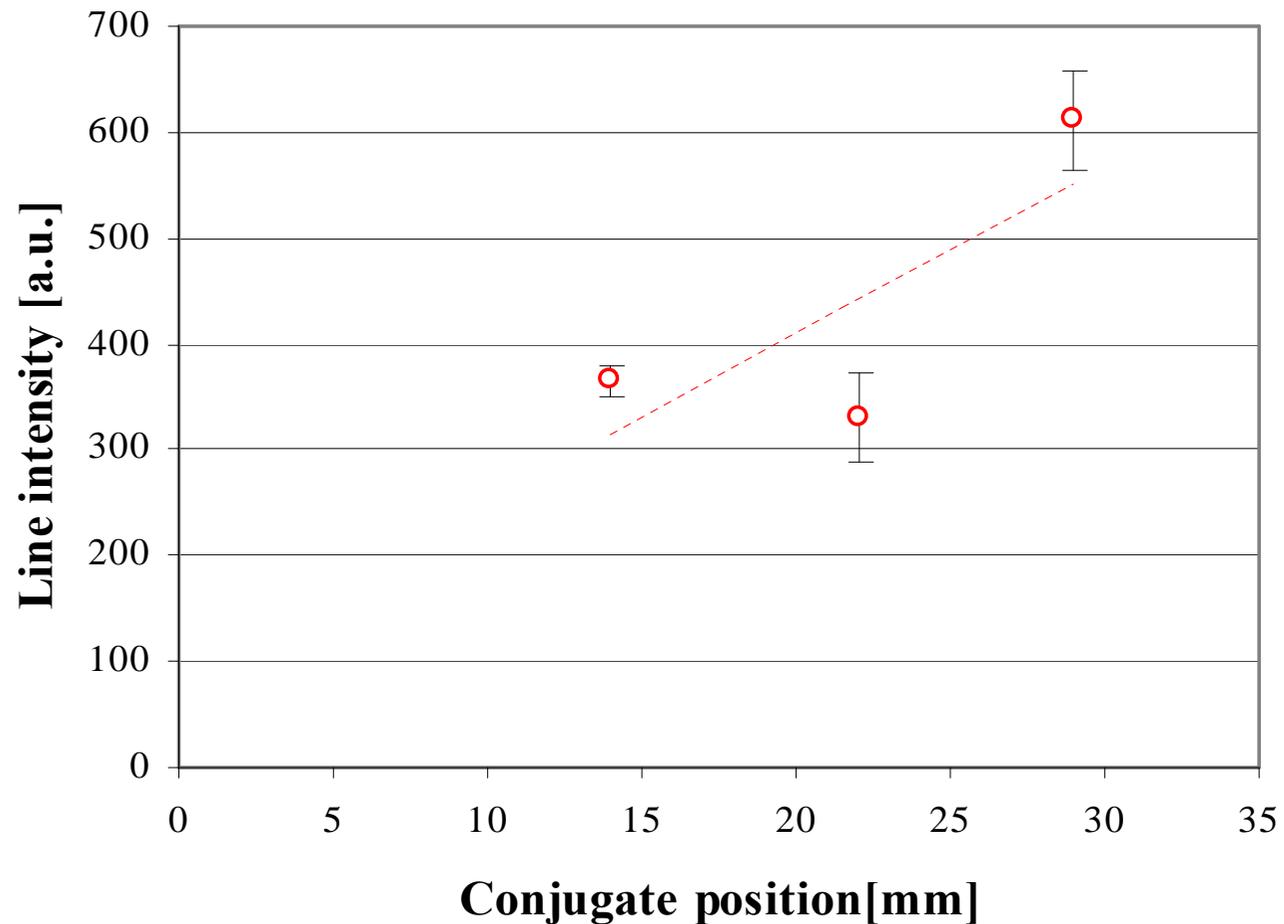
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Distance from release pad to test line

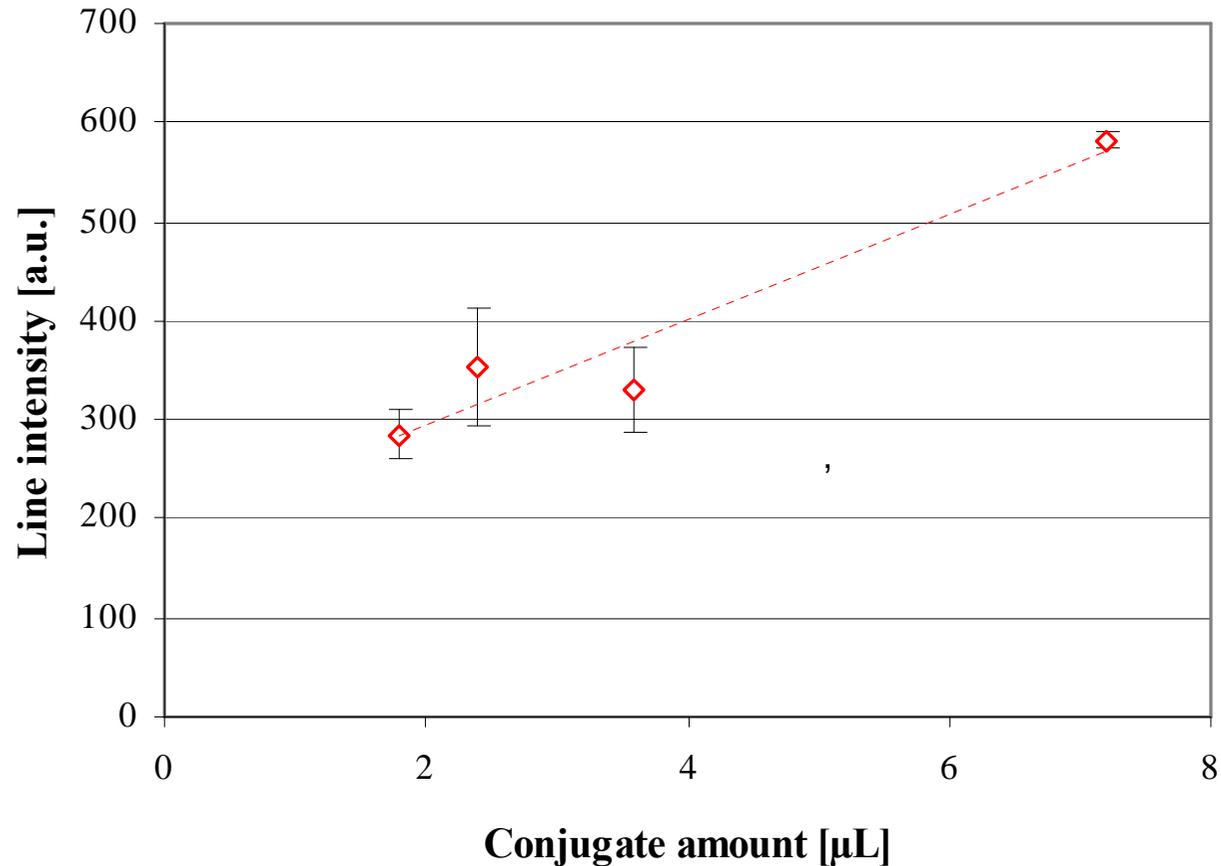


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Distance between conjugate and test line



Dependence on applied conjugate volume

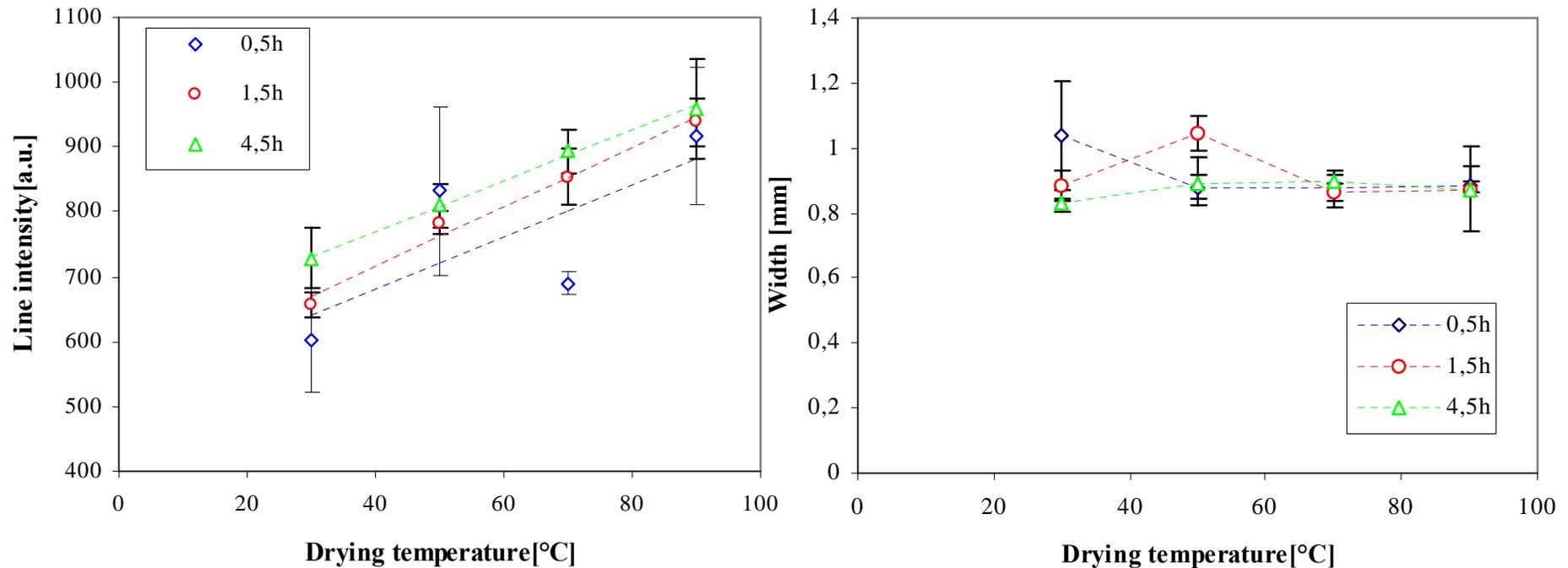


Influencing factors

=> membrane drying after striping

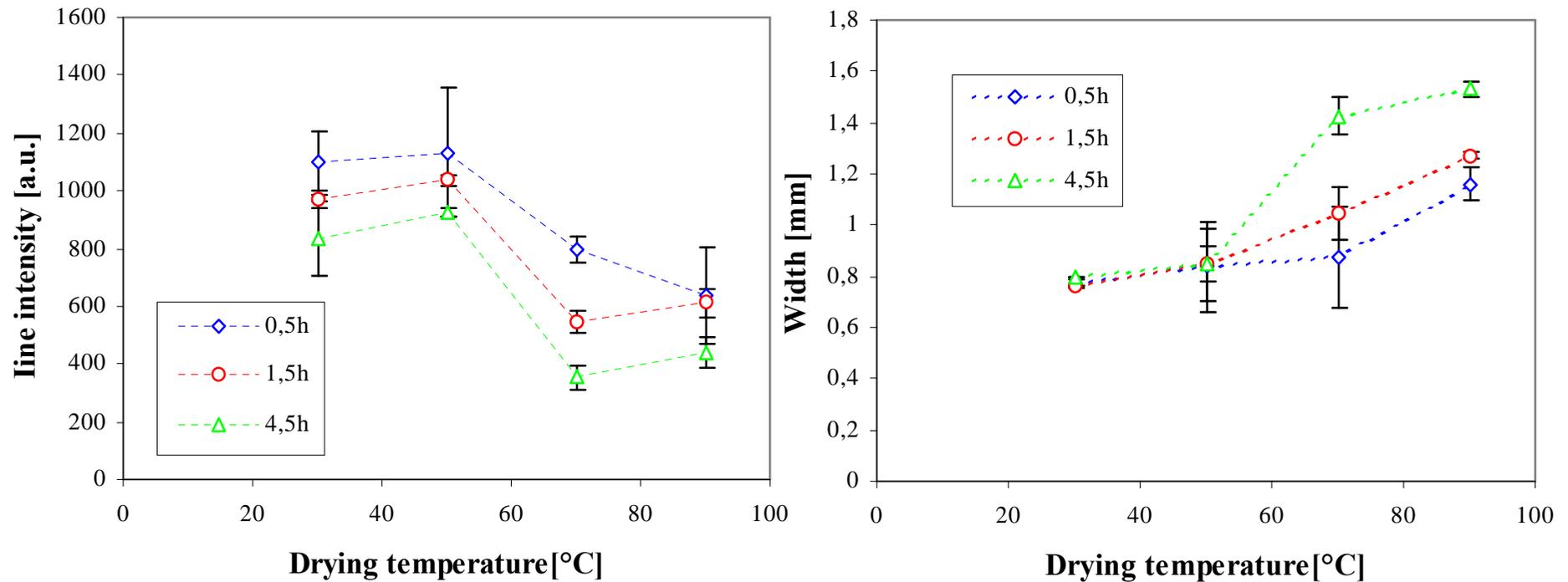
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Influence of drying time on test line: $t < 4.5h$

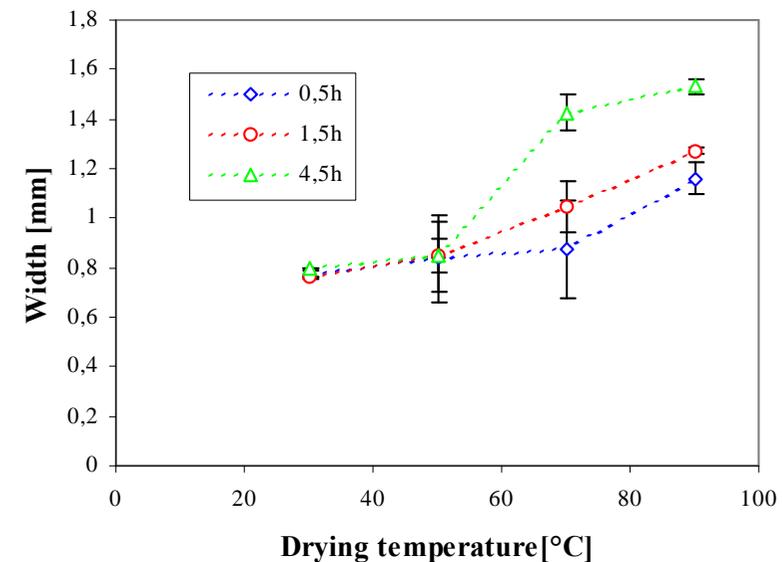
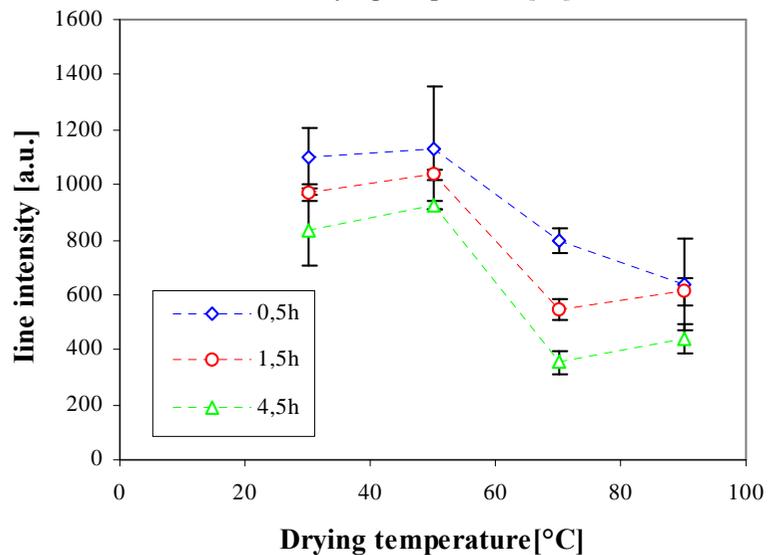
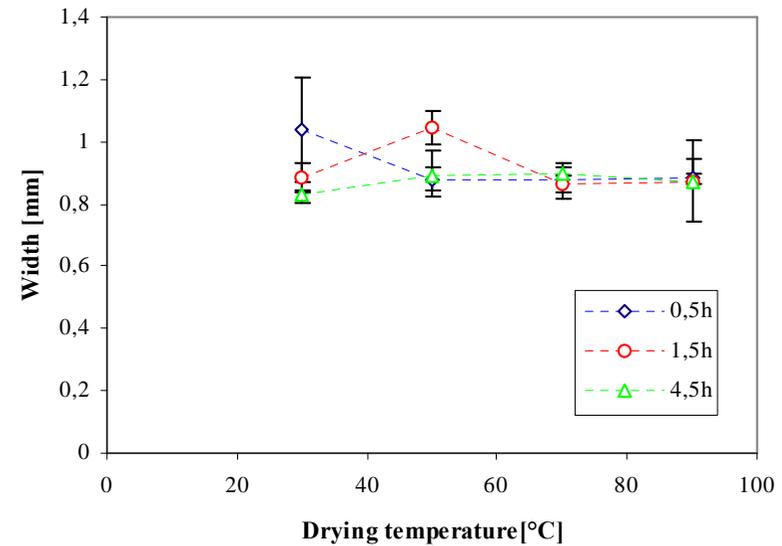
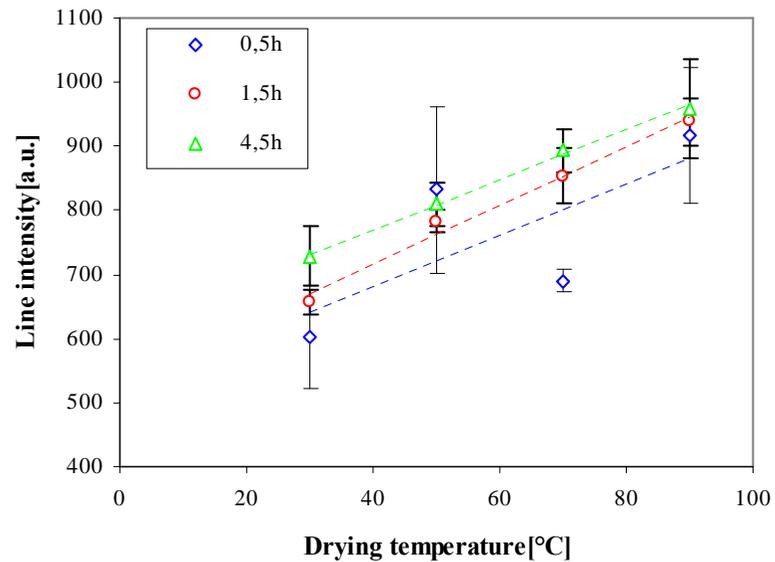


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Influence of drying time on control line: $t < 4.5h$

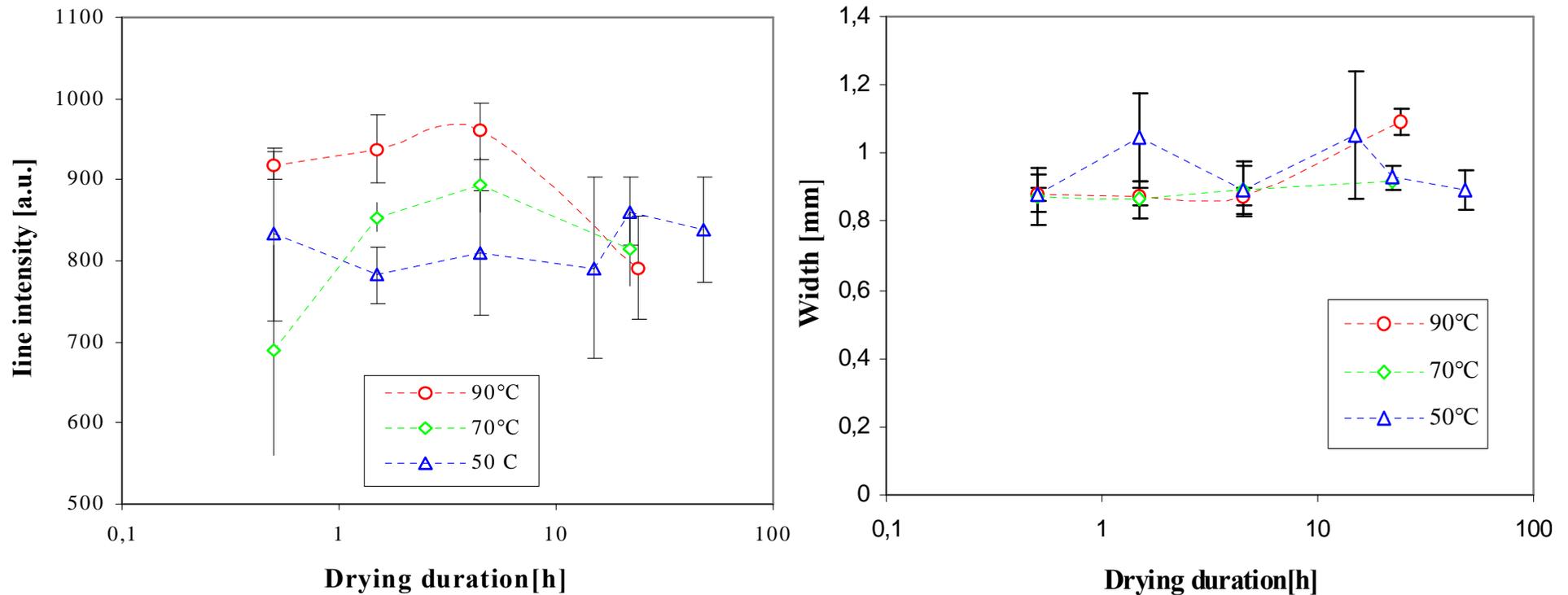


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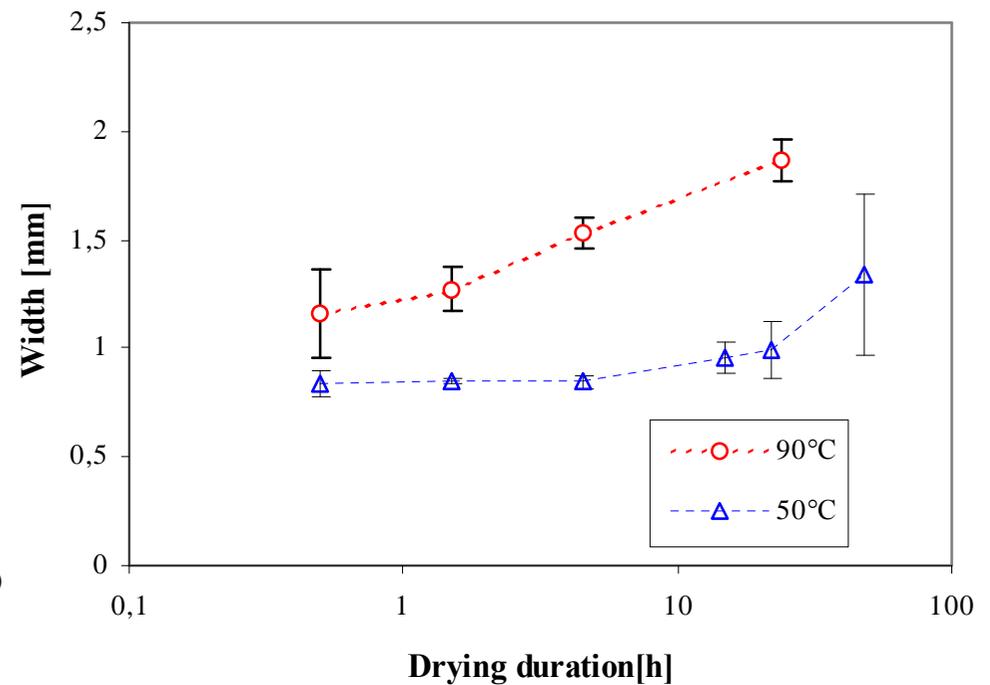
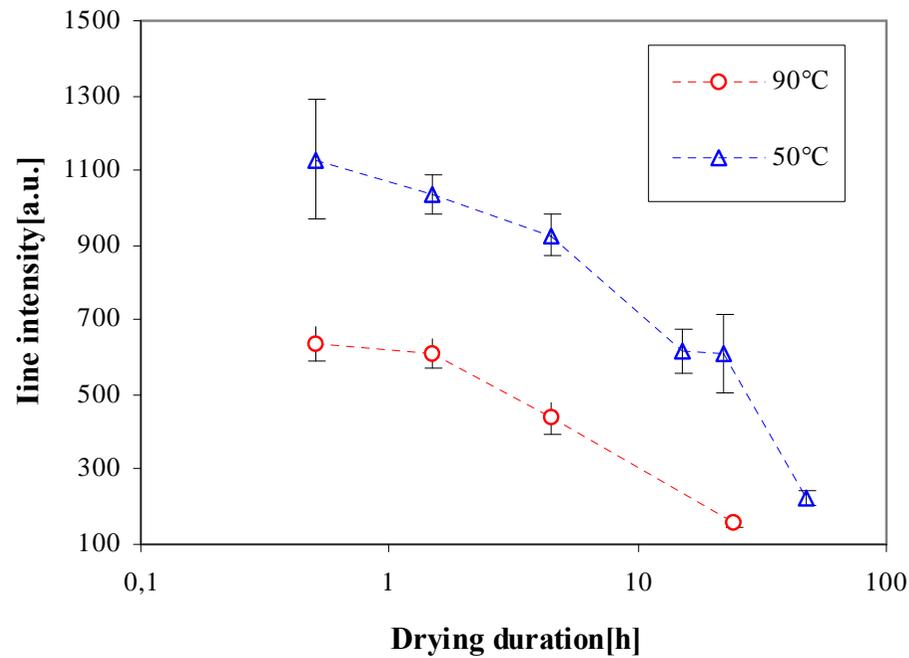
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Influence of drying time on test line: $t < 48\text{h}$

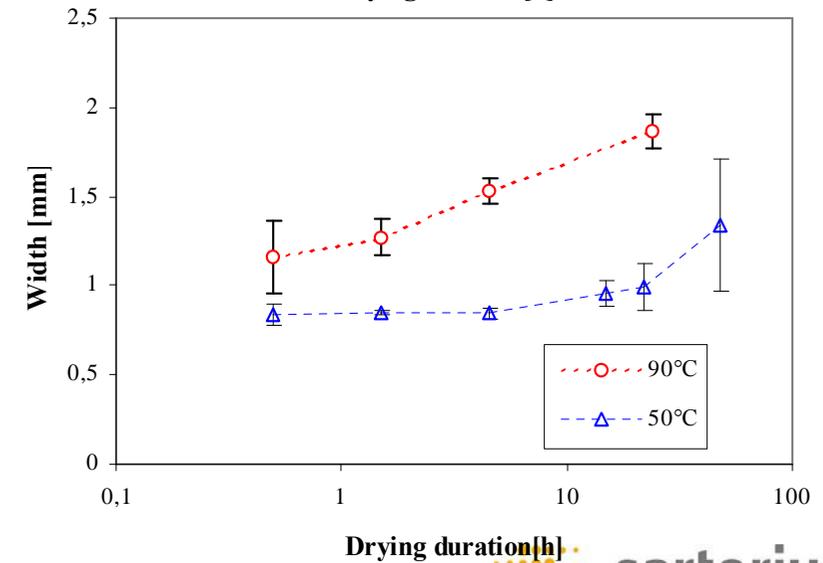
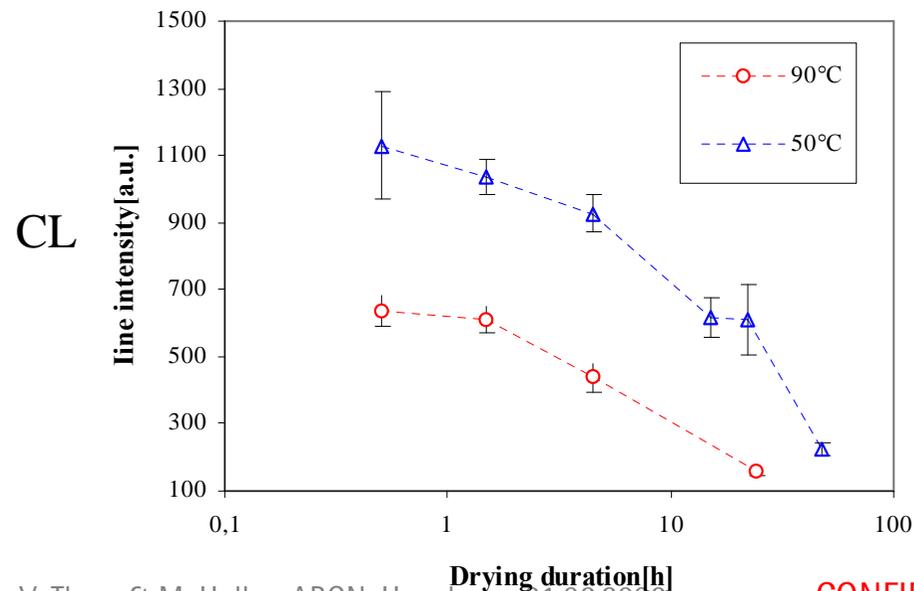
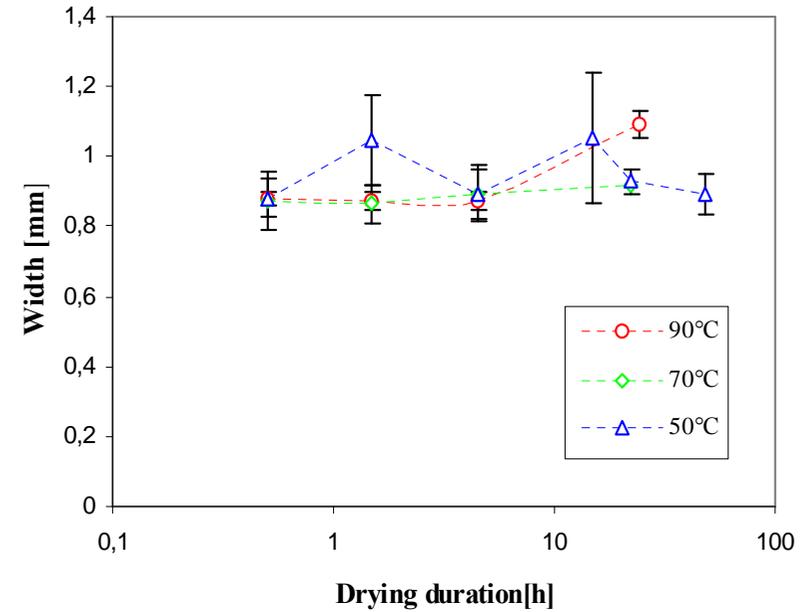
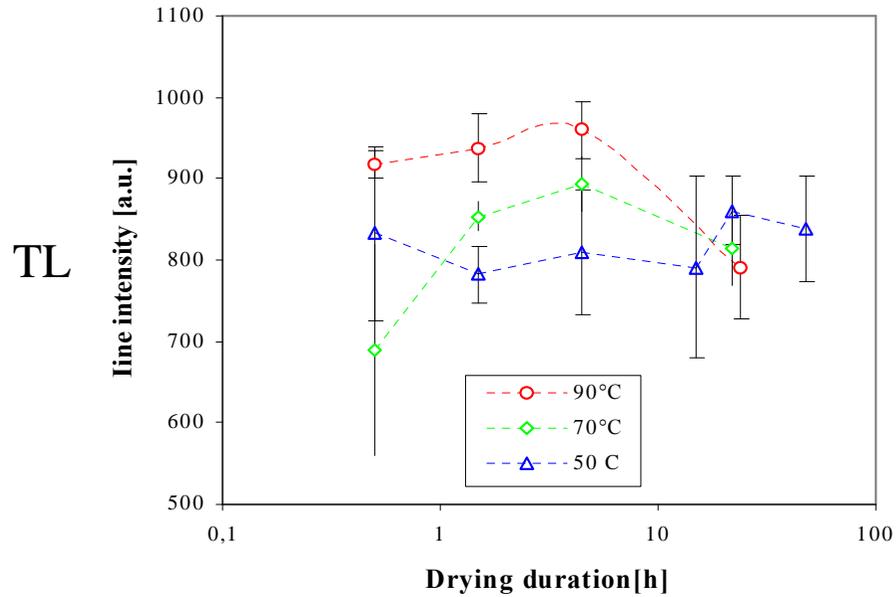


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Influence of drying time on control line: $t < 48\text{h}$

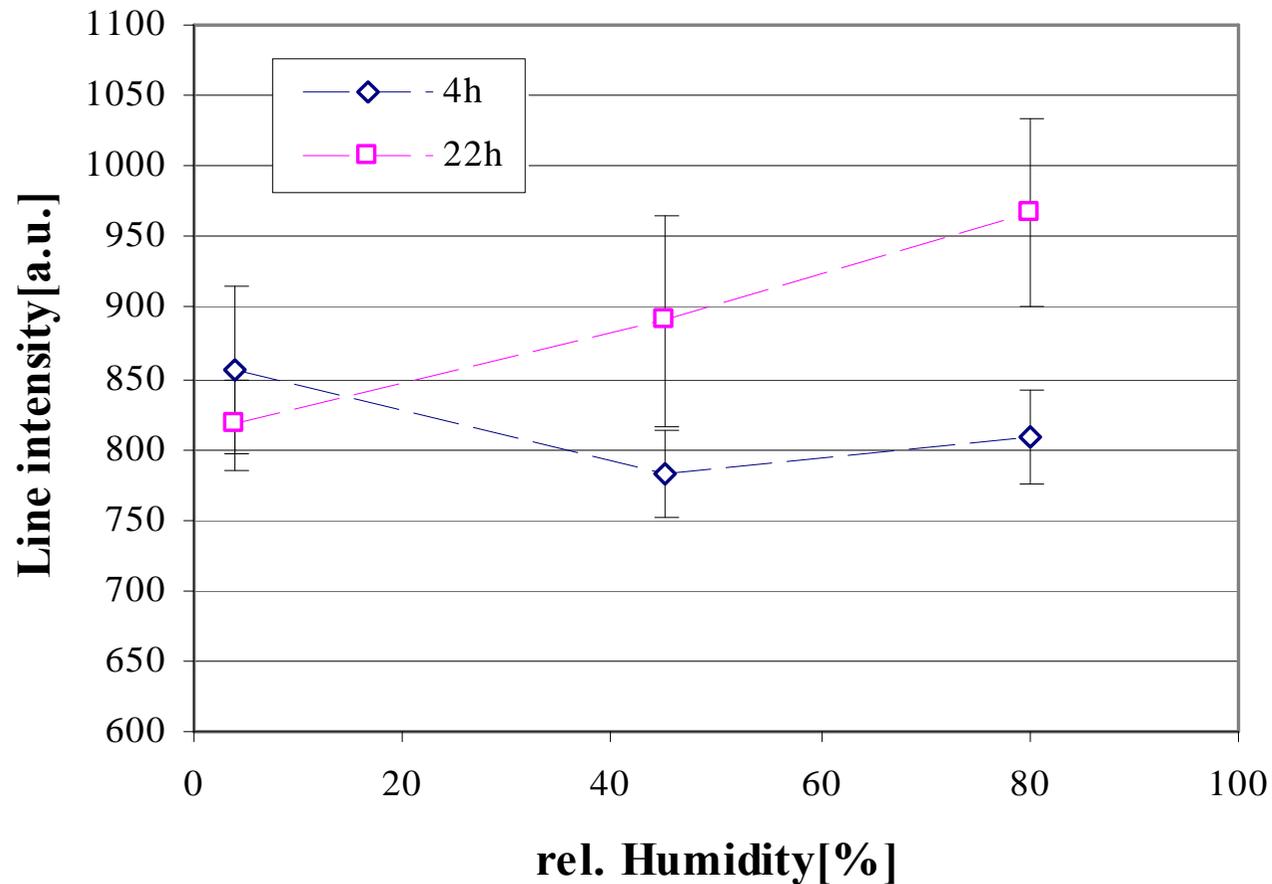


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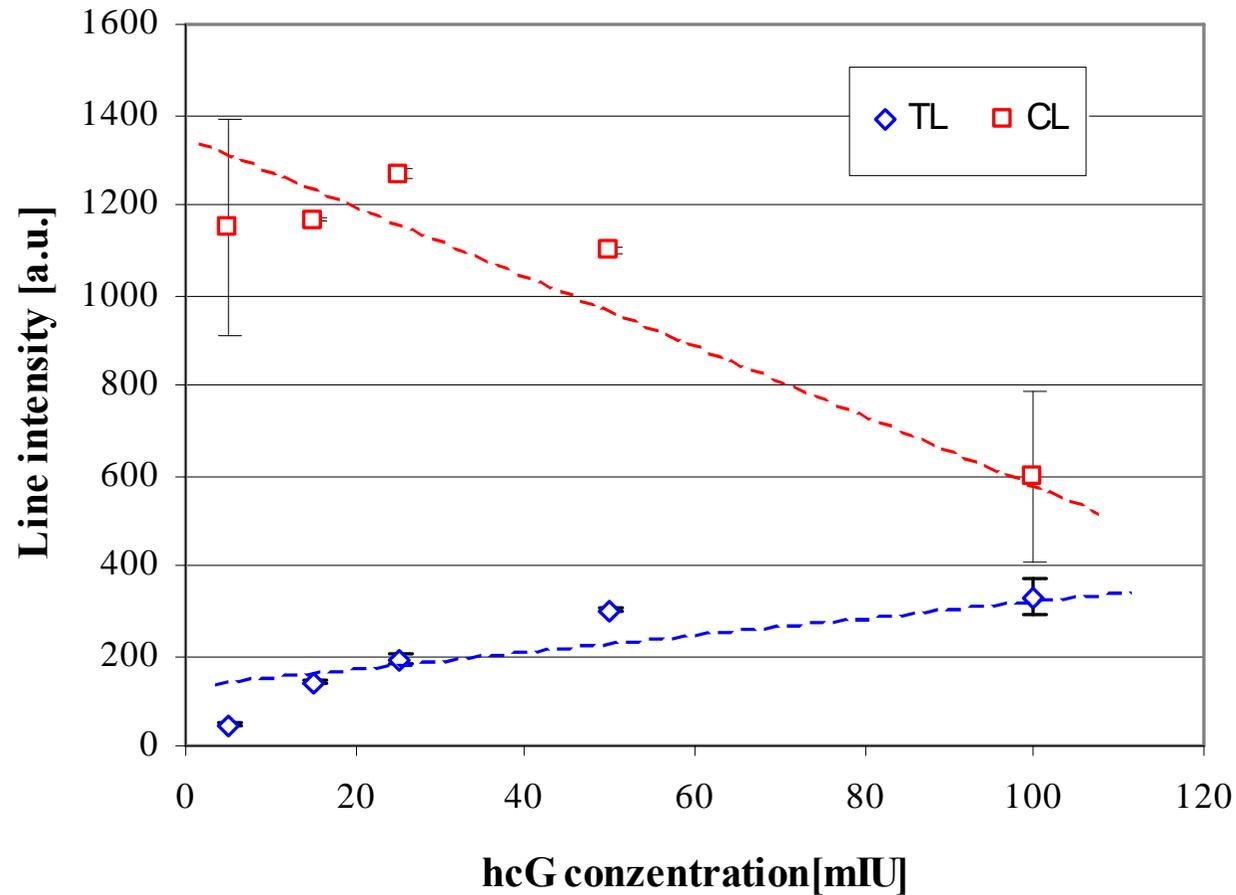
Influencing factors
=> running conditions

Influence of relative humidity



Test was equilibrated for a defined time at different relative humidities and run under the same conditions.

Dependance on the hCG Concentration



Conclusions

1. All investigated factors have considerable influence on test performance
2. Especially test architecture and drying conditions need to be consistent and validated to ensure robust test performance

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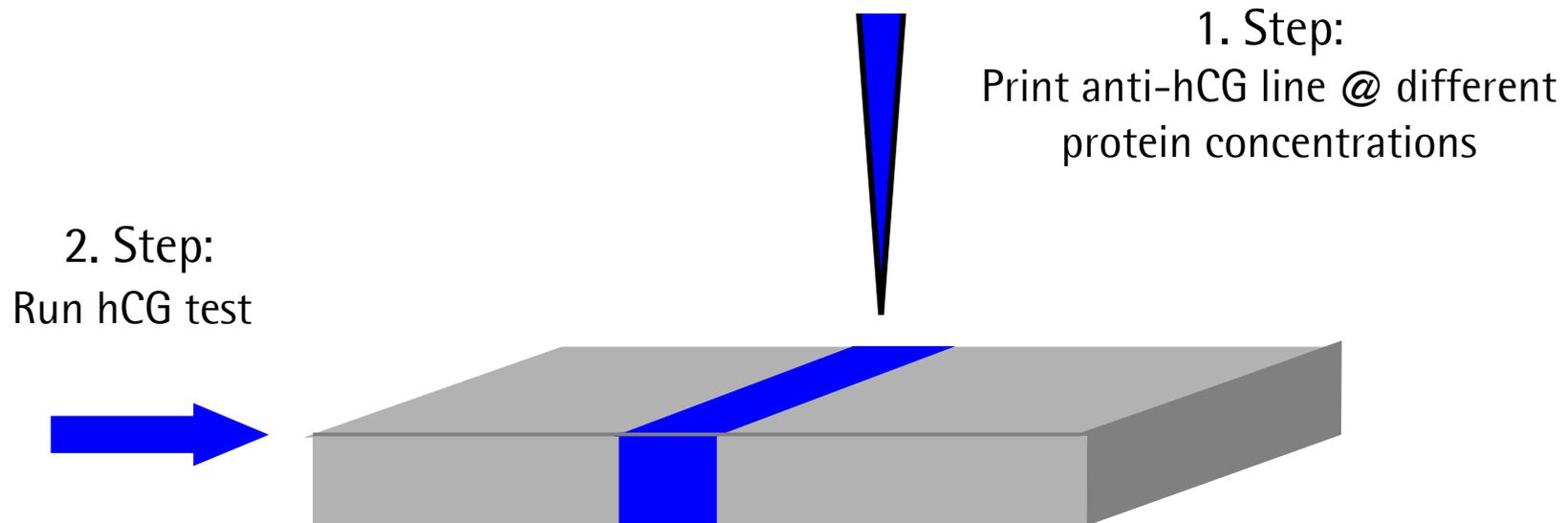
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Detection of capture antibody deposition through gold particle detection by vapor pressure SEM

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Experimental procedure:

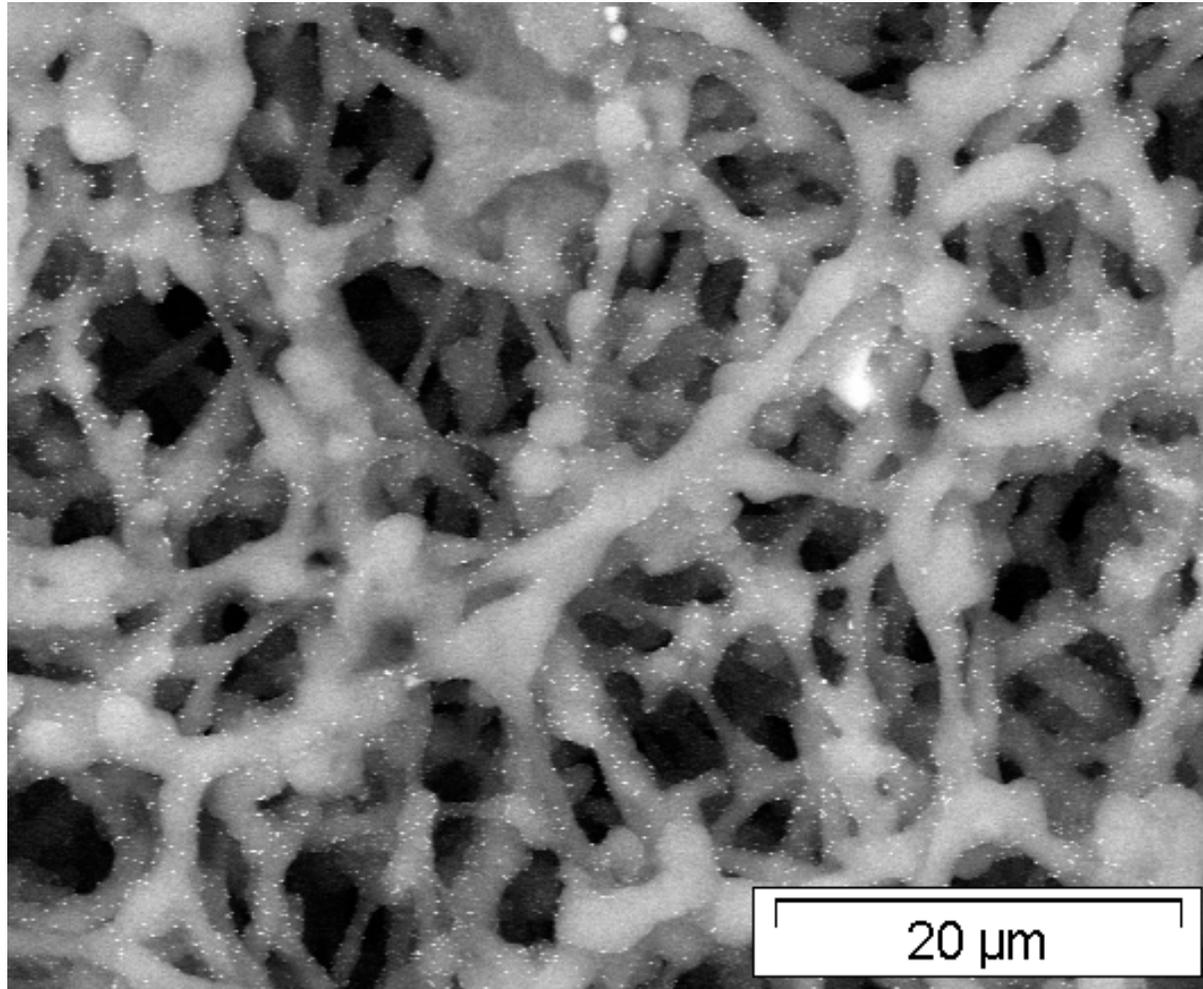


3. Step: Visualize gold conjugate on test-line cross-section by vp-SEM*

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Gold conjugate @ test line on a CN-membrane:

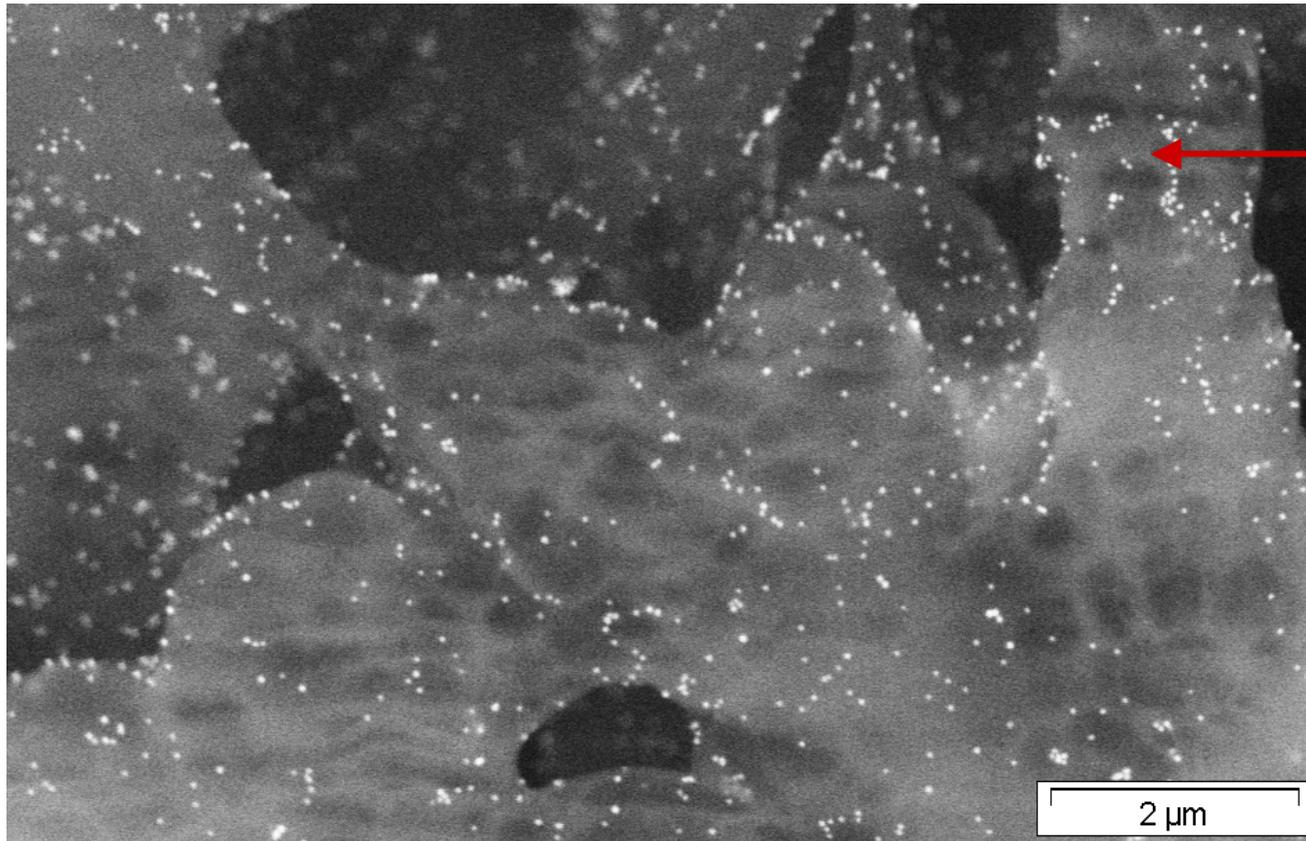
Technique: vapor pressure SEM without sputtering *



* vp-SEM was performed by Schossig, GKSS, Geesthacht, Germany

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=> higher magnification

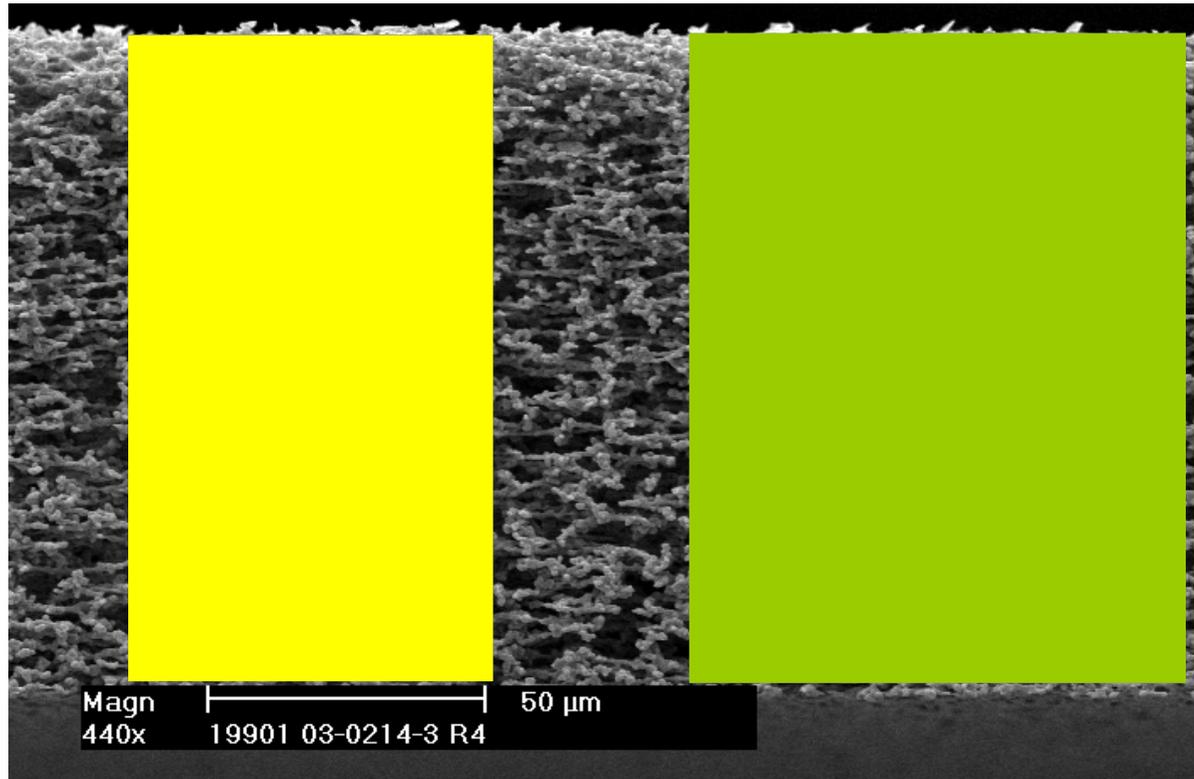


40 nm colloidal
gold particle

2 μm

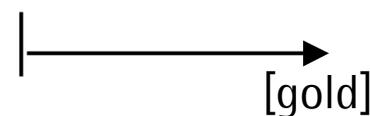
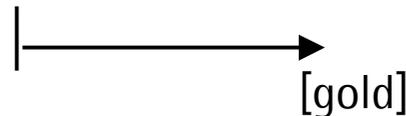
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Line printing at low antibody conc. generates anti-hCG gradient
=> detectable gold conjugate gradient (schematic)

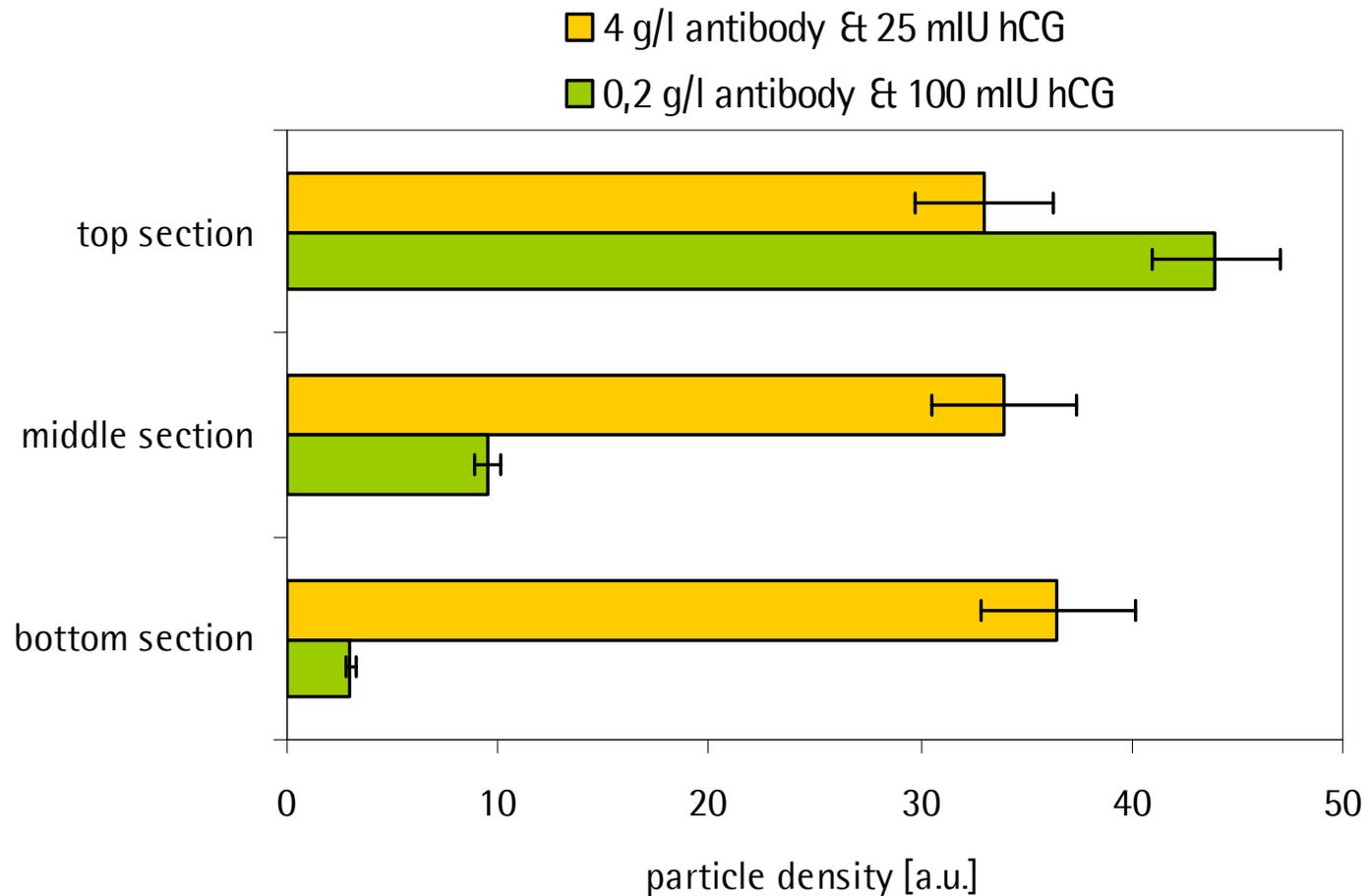


Anti-hCG = high conc

Anti-hCG = low conc



Line printing at low antibody conc. generates anti-hCG gradient
=> detectable gold conjugate gradient (actual data)



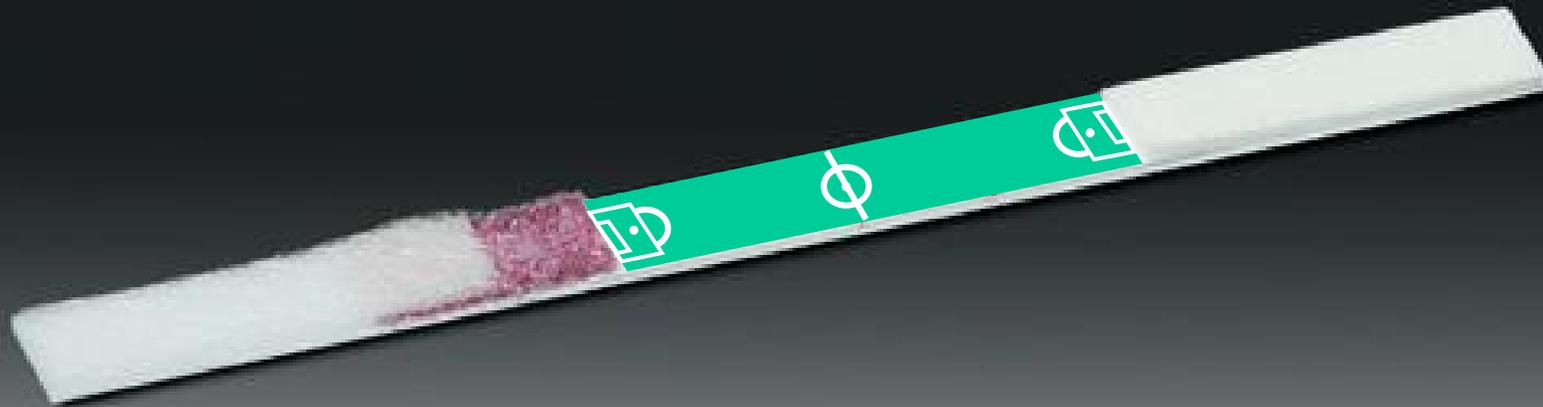
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Thank You!!



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Inverness Shanghai, Shanghai, 20.06.2006

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