Antibiotics and Nitrite detection in diagnostics with paper-based microfluidics

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Introduction

"Paper-based- microfluidics" (PBM) is a system that is considered low-cost, compact and an easy disposable technology. The main objective of PBM production is to keep it simple and reasonable.
The technical functionality of PBM is based on the capillary forces of cellular fibers within the paper. Nowadays, this behaviour is used in research and development to transport liquids microfluidic without the need of complex devices, e.g. pump systems.
In order to create reaction and detection zones on the paper, different methods are used to define these. Mostly hydrophobic barriers and hydrophilic areas are created to make assurance that reactants and samples come in contact. In addition, only small volumes of samples are needed to achieve valid detections.
Lately the field of food safety is growing, due to the fact, that the awareness for good quality food rises. Due to this, the area of food-monitoring with simple and reasonable technologies has risen. That's why researchers started to develop PBM based on validation of food quality. Some of these detectable substances are for example antibiotic residues and nitrite. Antibiotics, e.g. Streptomycin/Penicllin, are fed to many animals in order to keep the meat free from bacteria.

Objective

The aim of this project is to optimize the method of hot embossing by using different waxes and parameters, e.g. Template Temperature, Weights and Stamping Time, in order to obtain paper with the most remarkable qualities. With the use of this qualified paper, it is pursued to create an assay which makes it possible for the user to test his meat on nitrite and a mixture of Streptomycin and Penicillin. To reach this goal it is necessary to develop a method which allows to detect both substances on one PBM. The intention behind these objectives is to create a low-cost, resource-saving and user-friendly multi-assay based on paper.

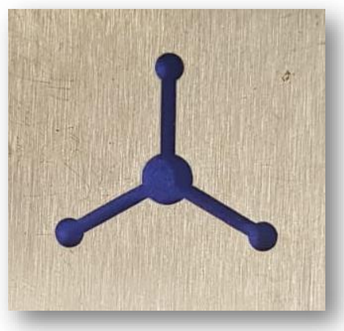
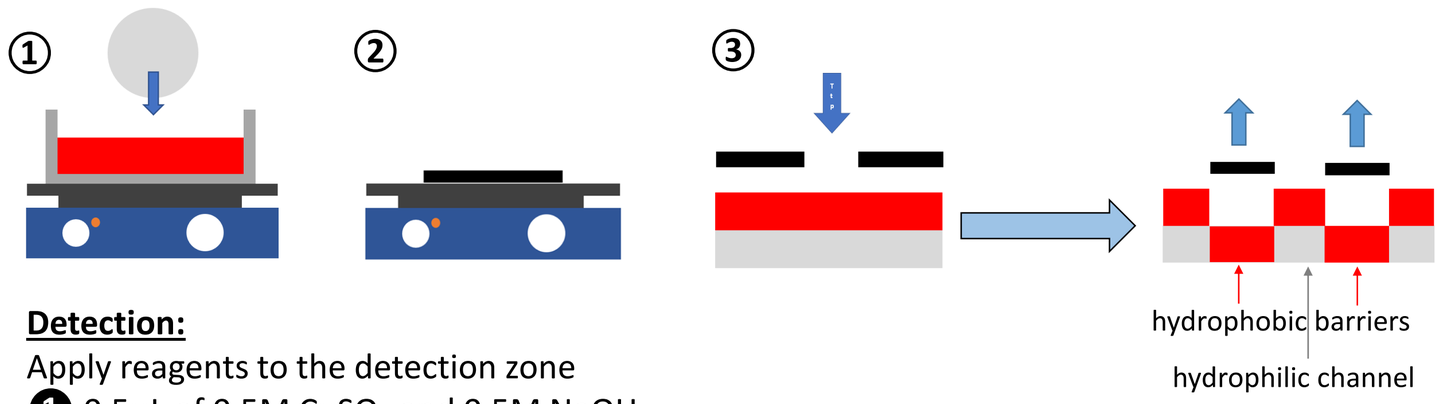


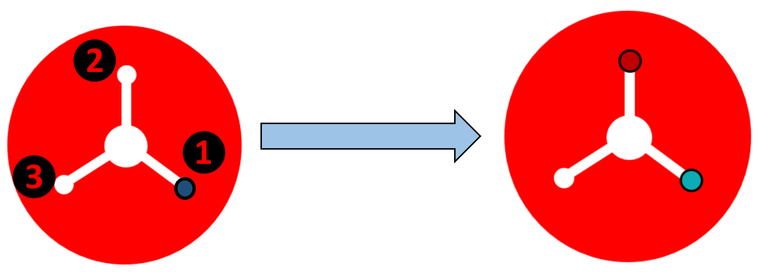
Fig. 1: Metal template
Channel width: 2 mm
Detection Zone: 12 mm^2
Sample Zone: 39 mm^2

Methods



Detection:
Apply reagents to the detection zone
❶ 0.5µL of 0.5M $CuSO_4$ and 0.5M NaOH
❷ 0,1µL of each Lunges A & Lunges B
❸ controle zone

Production:
① filter paper dipped in molten (red coloured) beeswax
② Heat metal template on heating plate
③ Hot Embossing
heated template placed on filter papers with different weights, stamping times and template temperatures



Results/ Discussion

In order to obtain the best PBM characteristics, beeswax showed the best features due to its low melting temperature and viscosity. Also different parameters were used to examine the impact on the channels and zones. In this test 1.8 kg, 30 s and 75 °C showed the most outstanding results in terms of accurate stamping and reproducibility. These results are based on statistical evaluations (see Fig. 2) which were carried out by photographing the individual samples and analyzing with Image J. The detection of nitrite and antibiotics showed positive results.
Nitrite was determined by using Lunges A & B which confirmed the presence of nitrite by a change from colourless to red. The detection of antibiotics was performed by using 0.5 M $CuSO_4$ and 0.5 M NaOH. The mixture of these reagents showed a blue precipitation and after adding the antibiotics the precipitation turned turquoise.

Moreover, the limit of detection for a stock solution of 10 000 µg/mL was determined, whereby the limit was 5000 µg/mL of antibiotics.
Furthermore it was tested whether a mixture of nitrite (1 M) and antibiotics (5000 µg/mL) disturbs any of the reactions or not. Fortunately, both reagents do not interfere with each other. With these information the required amount of reagents were tested. The principle „as little as possible but as much as necessary" was followed and led to the optimal amount of: Sample zone with 10 µL, detection zone of nitrite with 0.1 µL each, detection zone of antibiotics with 0.5 µL each.
To sum up, the hot embossing method was successful to create defined channels with beeswax and the parameters 1.8 kg, 30 s and 75 °C (see Fig. 3). Also the multi-assay of nitrite and antibiotics detection was successful (see Fig.4). Further steps would be to develop a method to test the assay directly on the meat.

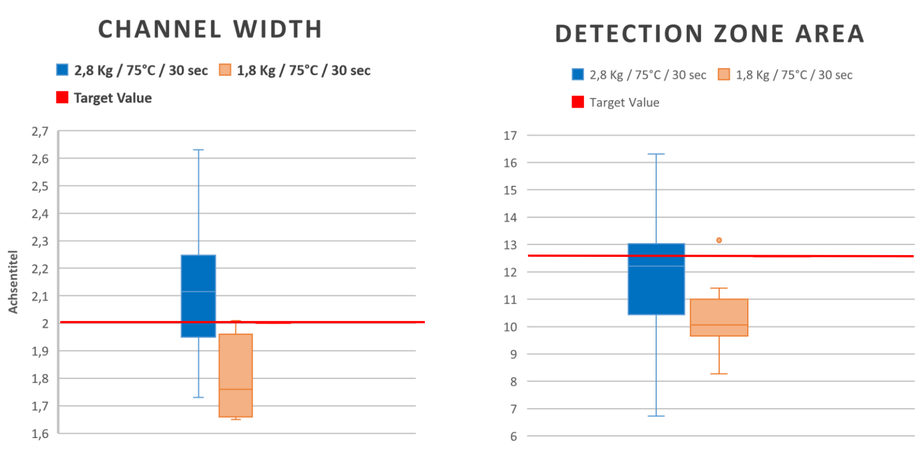


Fig. 2: Data Analysis of Channel width in mm (left) and Detection zone area in mm^2 (right)

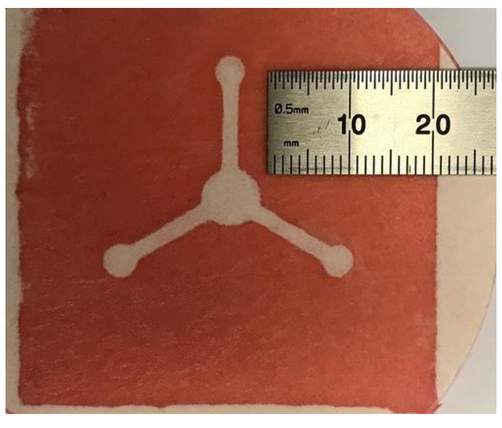


Fig. 3: Coloured Beeswax. Embossed with the parameters 1.8 kg, 30 s and 75 °C.

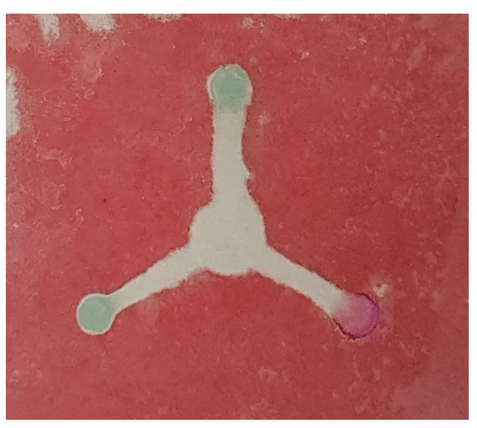


Fig.5: PBM with successful detection of nitrite and antibotics

Referenzen: https://pubs.rsc.org/en/content/articlelanding/2015/ay/c5ay00466g#!divAbstract; https://www.ncbi.nlm.nih.gov/pubmed/30482295