STABLE DRIED ENZYME-LOADED POROUS STRUCTURES FOR POINT OF CARE DIAGNOSTICS

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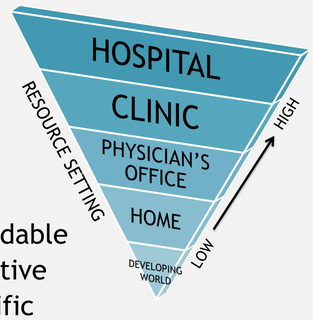
MOTIVATION

POINT OF CARE (POC) TESTING

Medical testing performed at or near the site of patient care.



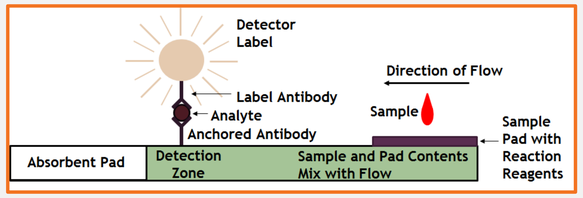
LOW RESOURCE SPECIFICATIONS:

World Health Organization

Affordable
Sensitive
Specific
User-friendly
Robust and rapid
Equipment free
Deliverable to those in need

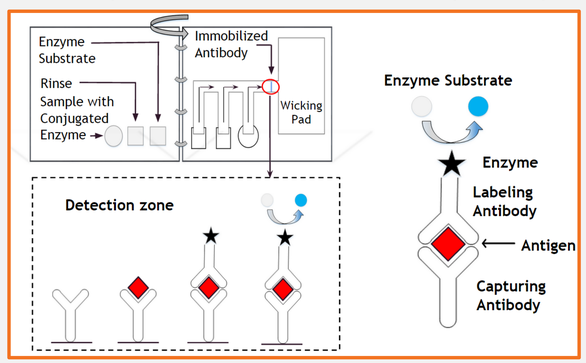
LATERAL FLOW TESTS

Lateral flow tests have been used for decades to address testing needs in POC settings. However, they offer only yes/no responses and are limited by a single chemical processing step.



PAPER MICROFLUIDICS

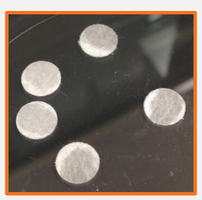
Paper microfluidics offers multi-channel capability that can be used for signal amplification. This allows improved limits of detection and specificity.



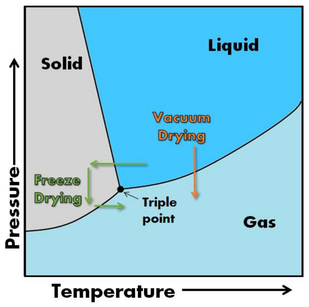
OPPORTUNITY

Improve POC microfluidic tests:

- Increase *ease of use* via source pads pre-loaded with *dry* reagents
- Increase enzyme *shelf life* in source pads via drying with lyoprotectants



DRYING METHODS

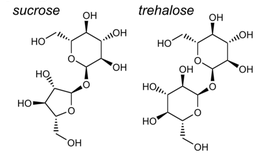


Freeze drying, or lyophilization, takes advantage of the process of sublimation. First samples are frozen to -80 °C, and then they are placed in a 50 mTorr vacuum chamber in which ice crystals change directly into water vapor, bypassing the liquid phase. Vacuum drying evaporates water by reducing pressure, leaving dried reagents in samples. These methods generally increase shelf life of perishable materials, and makes transport easier.

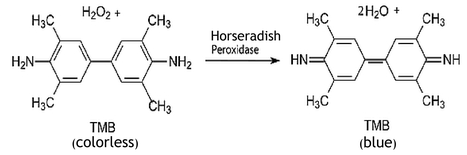
Lyophilizer in Magruder Hall at OSU

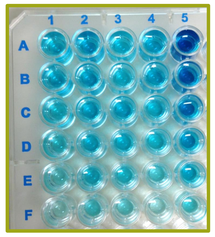
LYOPROTECTANTS

Lyoprotectants are components that help preserve the structure of biomolecules during drying.



ASSAY





FORMULATION COMPONENTS

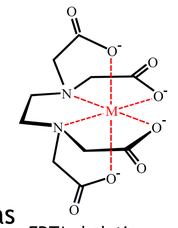
HRP: The enzyme horseradish peroxidase is commonly used in immunoassays, as it has convenient chromogenic substrates.

Sucrose or Trehalose: Non-reducing sugars resist crystallization and help support the protein as water is removed.

Structure of horseradish peroxidase

Metals: Polyvalent metal ions appear to exhibit a stabilizing effect on HRP during freeze drying. Our team is testing $FeSO_4$ and $MgSO_4$.

EDTA: This compound is used as a metal sequestering agent.



EDTA chelation of a metal ion

RESULTS

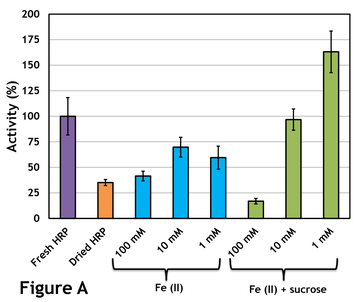


Figure A

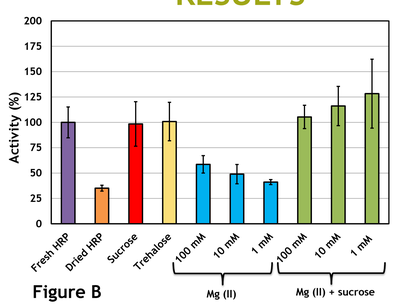


Figure B

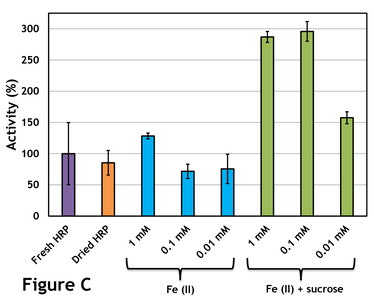


Figure C

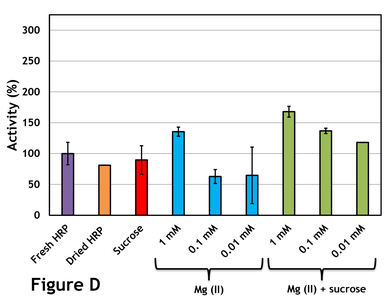


Figure D

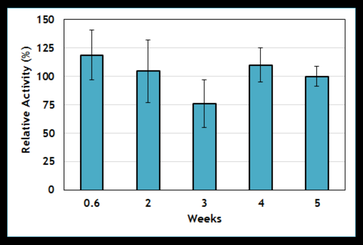
These figures display HRP activity with various added lyoprotectants compared to fresh (purple) and dried HRP-only standards (orange).

Figure A displays freeze dried formulations with iron and sucrose additives.

In **Figure B**, freeze dried formulations with sugars and magnesium formulations are shown.

Figure C shows vacuum dried formulations with iron and sucrose additives.

In **Figure D,** vacuum dried formulations with magnesium and sugar additives are displayed.



In **Figure E**, HRP activity does not degrade significantly over five weeks when stored at high concentrations in solution.

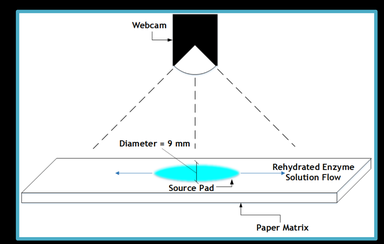
Figure E: HRP activity over a five week period within 90% confidence.

CONCLUSIONS

- Sucrose and trehalose appear to equally preserve HRP activity through the freeze drying process.
- Metals potentially inhibit the HRP reaction with TMB at higher concentrations.
- Combinations of additives provide better preservative effects compared to individual components.
- Data inconsistencies between experiments suggests uncontrolled conditions are present.

FUTURE WORK

- Develop protocol for controlled drying of enzymes and other reagents in paper matrices.
- Investigate TMB assay to increase reproducibility and reduce variability in HRP activity measurements.
- Test Fe(III) as a lyoprotectant to determine if enzyme preservation is charge specific.
- Characterize HRP release profile out of glass fiber pad into paper matrix.



Webcam method for observing enzyme release from source pad. MATLAB analysis can model enzyme quantity exiting pad over time.

ACKNOWLEDGEMENTS

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