At the Crossroad of Biology, Chemistry, and Machine Learning: Catalase Assay by Iodine Clock Reaction

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INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

The beginning

Hi Takao, I need a method to measure catalase activity, but I have no external funding for my research.

You are the biochemist, you'll figure out something cheap and reliable!

Okay, let's see what can I do four you!

An inspiration

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:

TECHNIQUES AND APPLICATIONS

BIOCHEMICAL ASSAYS

A Simple Assay for Measuring Catalase Activity: A Visual Approach

Tadayuki Iwase[1], Akiko Tajima[1], Shinya Sugimoto[1], Ken-ichi Okuda[1], Ippei Hironaka[2], Yuko Kamata[3], Koji Takada[4] & Yoshimitsu Mizunoe[1]

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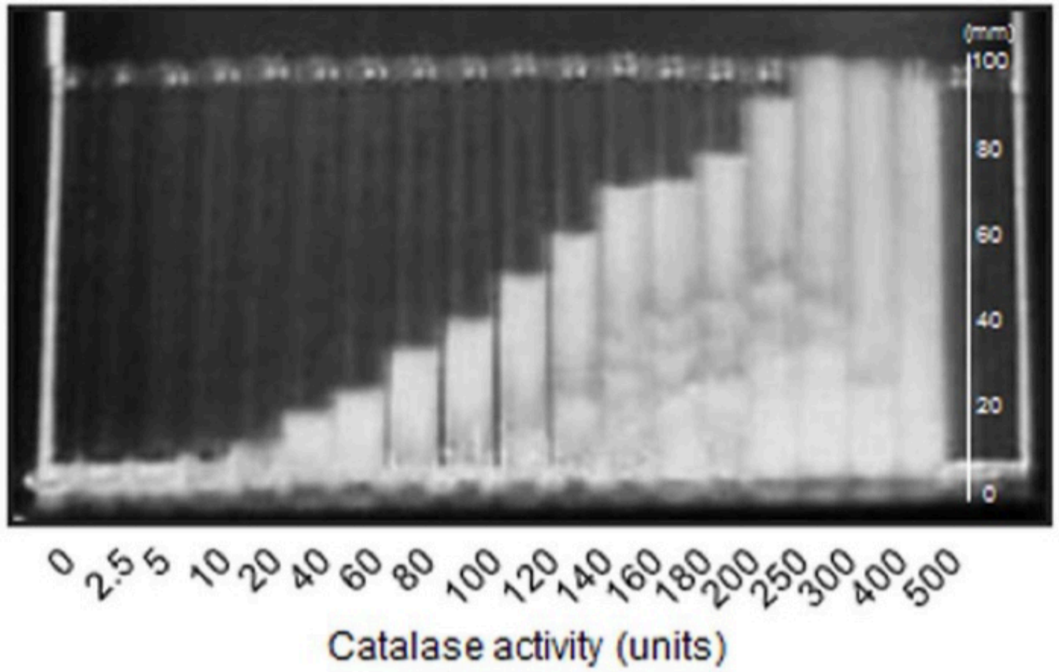
inhibitor azide was used and, as expected, foam form
inhibited.

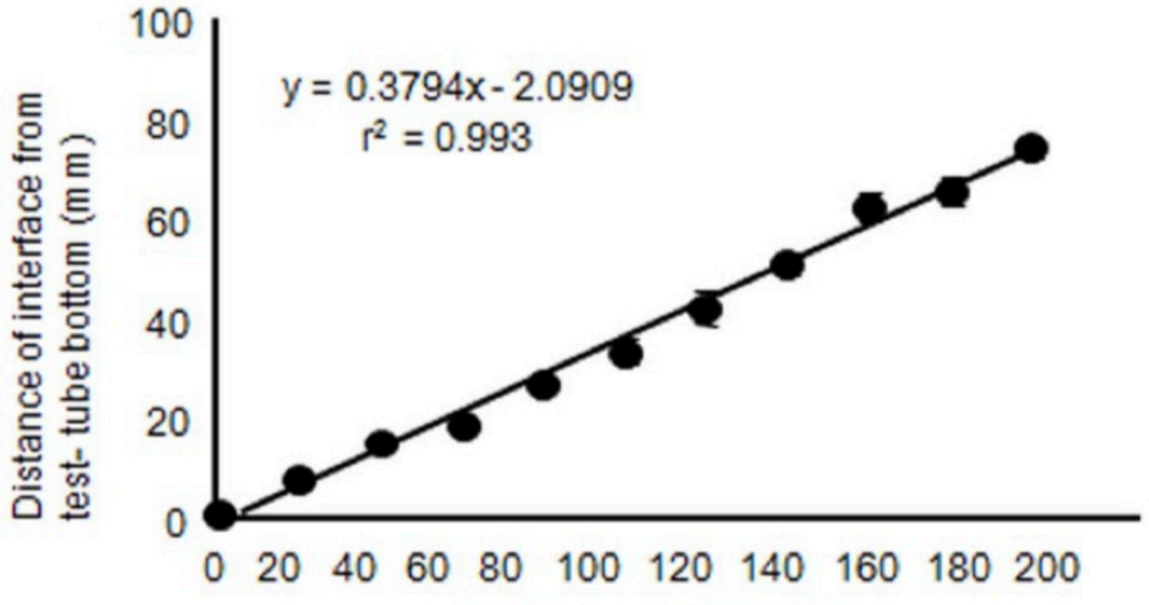
By applying the developed method to *E. coli* strains carr
tions in the catalase genes or in their regulatory factors, w
to determine the catalase activity in the *katE*, *katG*, and *rp*
mutants, as well as in the wild type (Fig. 2A), and to demo
ability of the assay to accurately discriminate between the
HPI and HPII. Only HPI or HPII activity was observed
and *katG* deletion mutants, respectively, and the *rpo*
mutant showed only HPI activity. In contrast, both HP
activities were noted in the wild-type strain.

Furthermore, different clinical isolates were analyse
catalase activity (Fig. 2B). HPI activity was detected in all

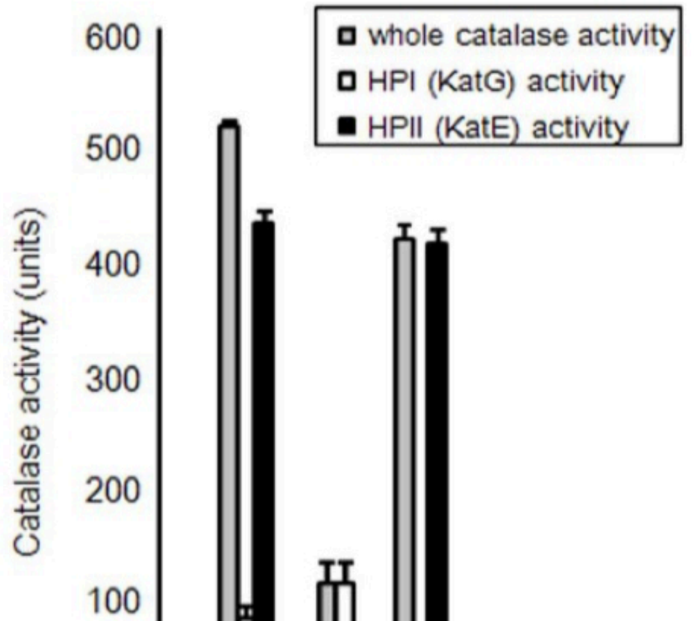
A



B

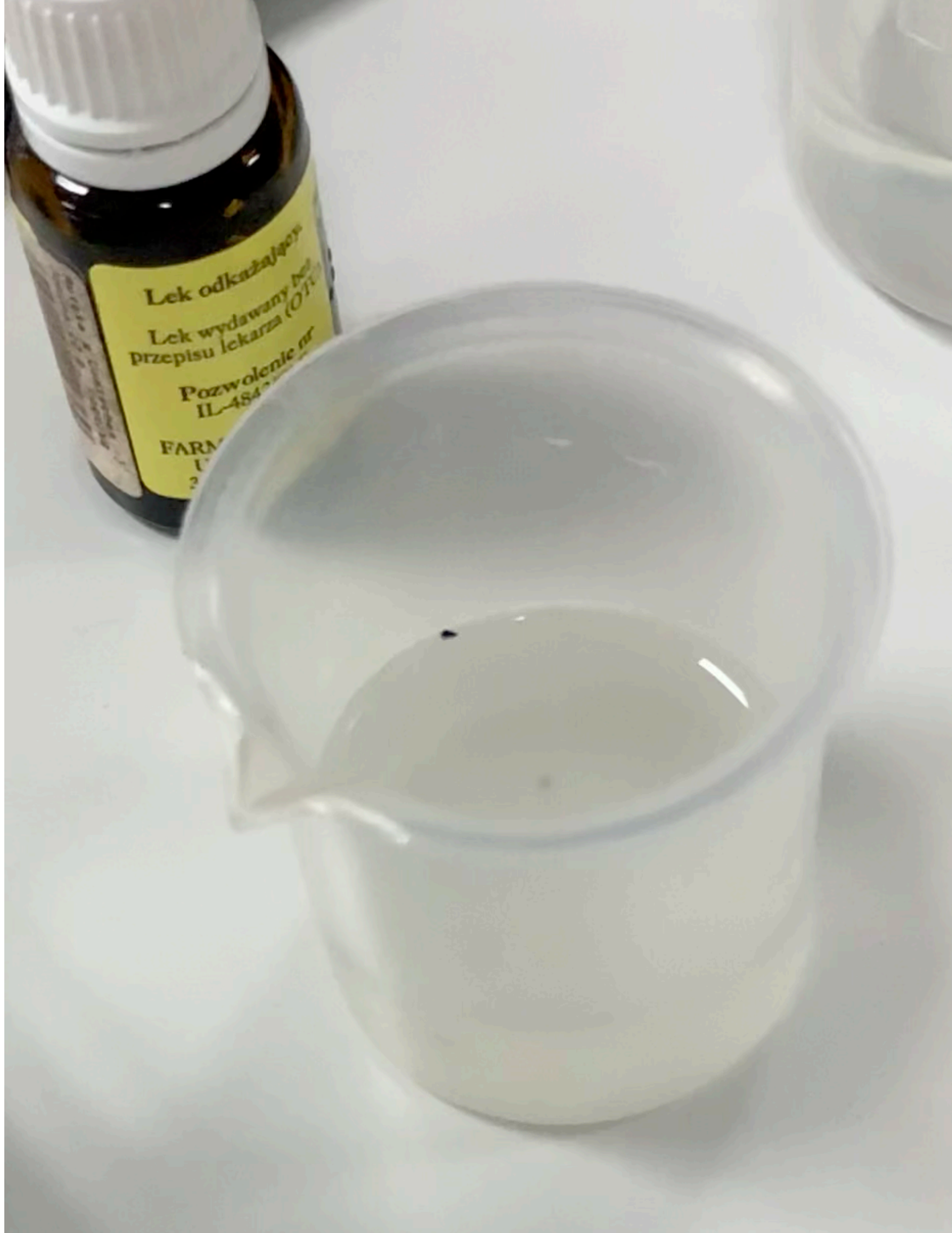


A



Iodine clock reaction

- 1 ml iodine weak solution (2–3%)
- slowly add 2 ml ascorbate solution (0.2%)
  - dark brown colour changes to light yellow
- add 0.8 ml starch solution (1.0%)
- add 8 ml water
- add 8 ml hydrogen peroxide (3%)
- just wait for a while (a few minutes)



The chemistry behind

Catalase Assay by Iodine Clock Reaction – CAICR

reduction

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I^- + 2H^+$$

(ascorbate) (ascorbate)

oxidation

$$2I^- + H_2O_2 + 2H^+ \rightarrow I_2 + 2H_2O$$

=

catalase: $H_2O_2 + H_2O \rightarrow 2H_2O + O_2$

So, what is the point?

Catalase Assay by Iodine Clock Reaction – CAICR

- **high catalase activity**
- less H_2O_2
- less I_2
- longer time required for the regeneration of I_2, thus the colour reaction with starch will be delayed

slow

- **low catalase activity**
- more H_2O_2
- more I_2
- shorter time (without delay) required for the colour reaction of I_2 with starch

fast

0

colour change solution
(C solution; iodine + ascorbate + starch)

178 µl

2

10 min. reaction

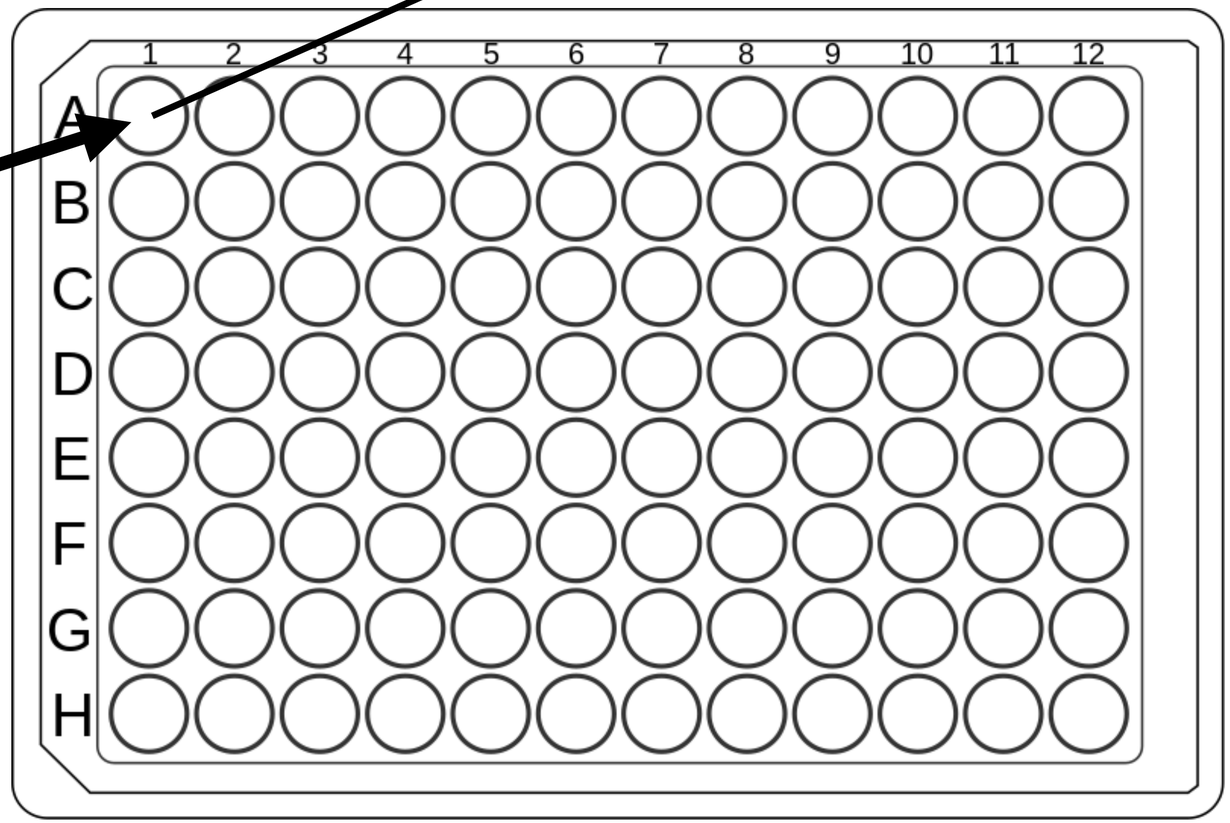
H_2O_2 30%

1

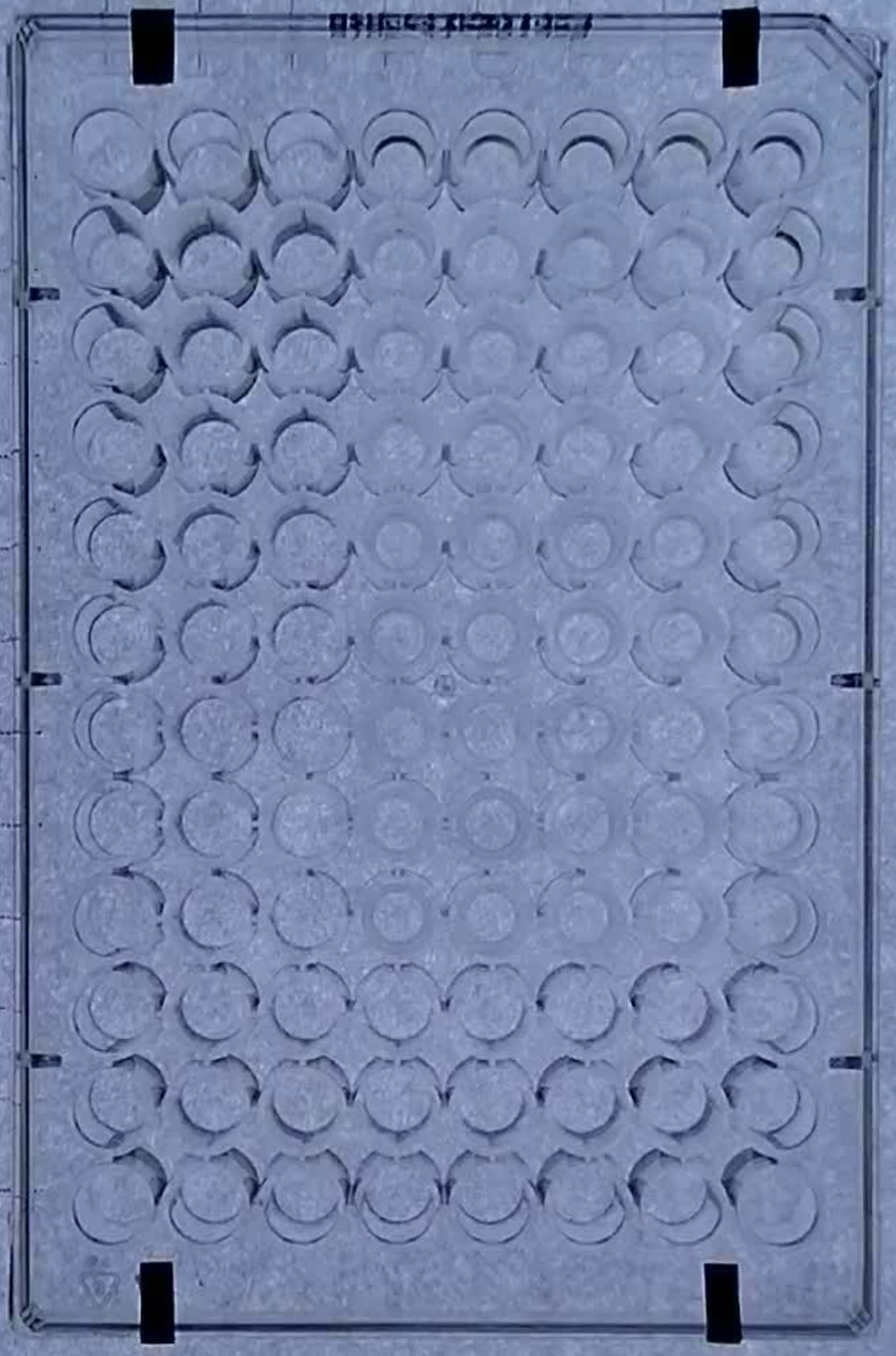
catalase

80 µl

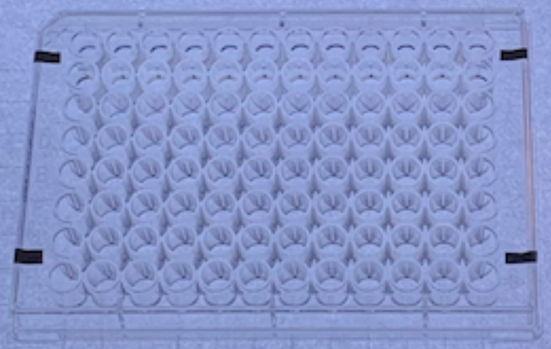
3



enzymatic reaction solution
(E solution; catalase + H_2O_2)

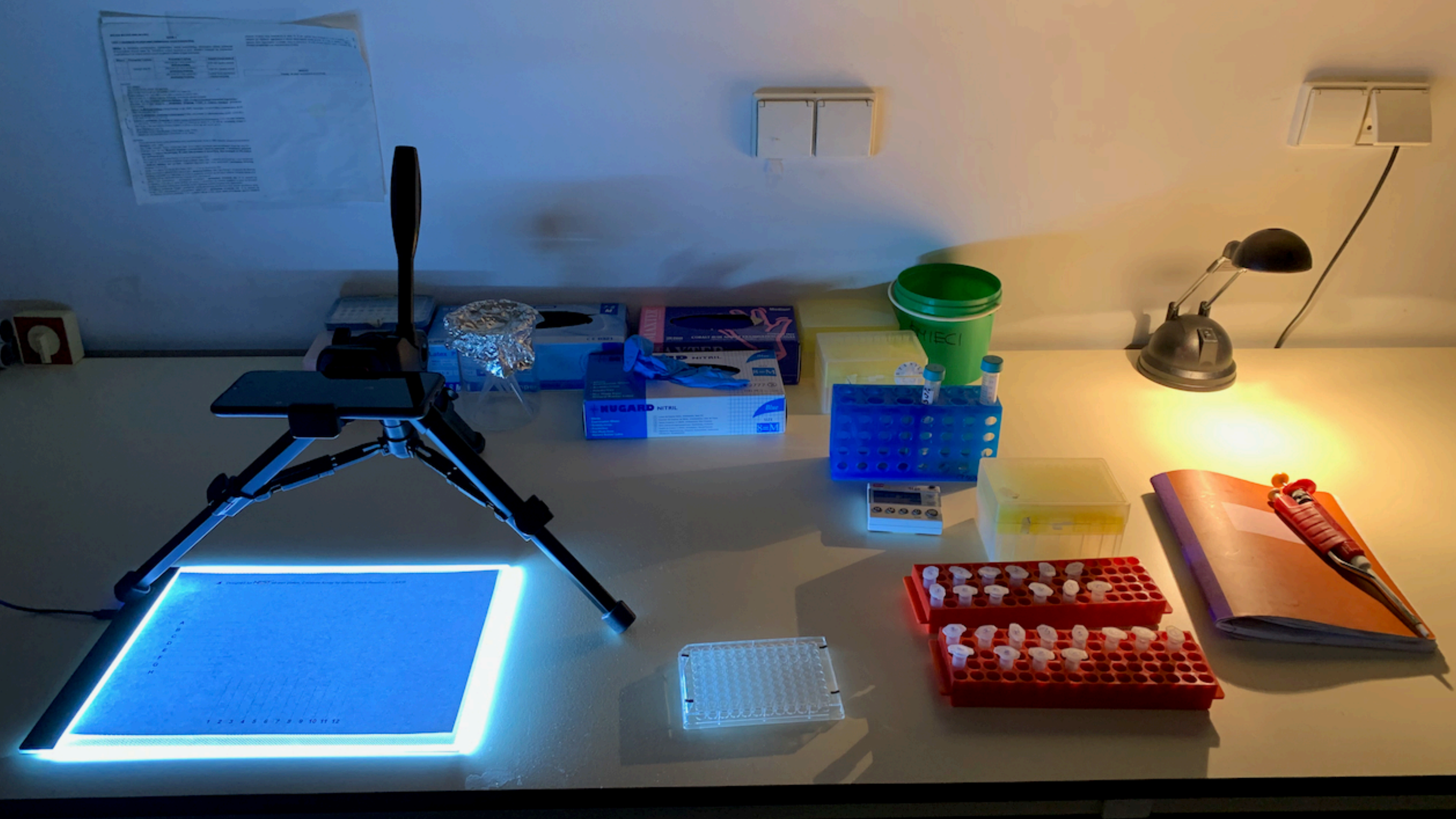


Designed for NEST 96-well plates. *Catalase Assay by Iodine Clock Reaction – CAICR*

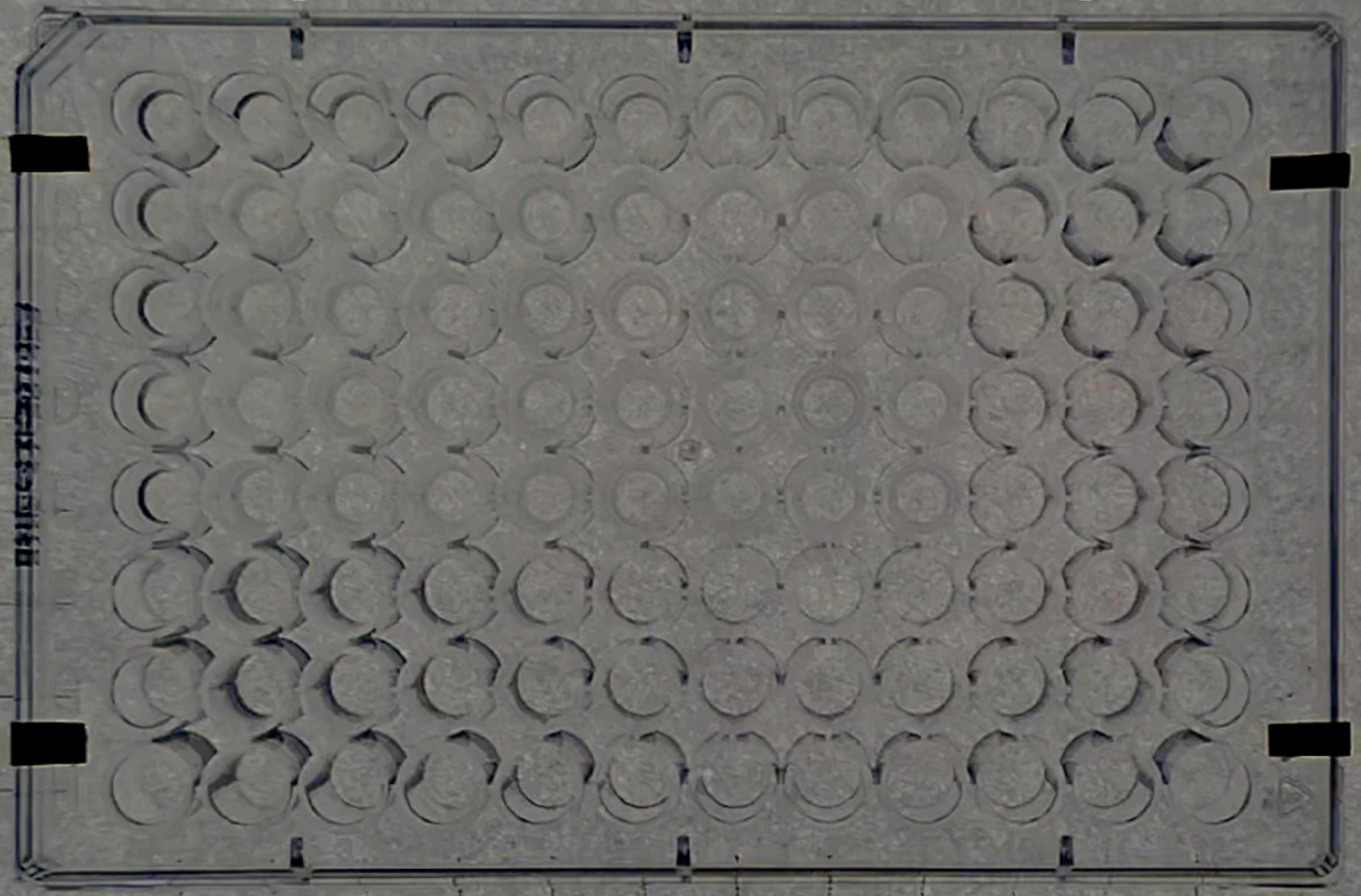


A
B
C
D
E
F
G
H

1 2 3 4 5 6 7 8 9 10 11 12


HUGARD NITRIL

20 min. experiment shown in 1 min. (20×accelerated)

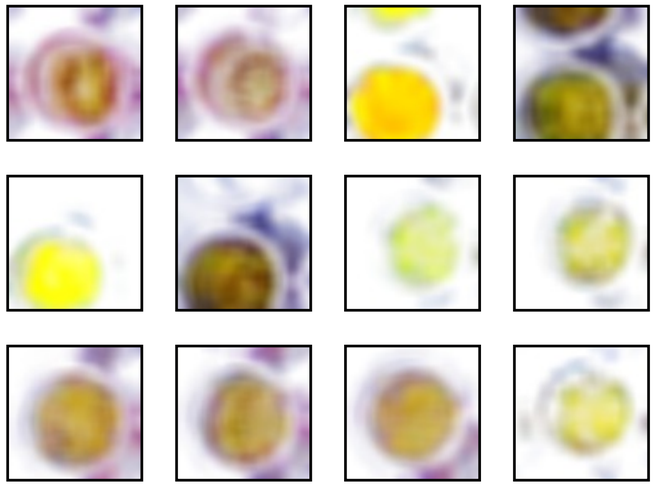


How to identify the reaction as completed?

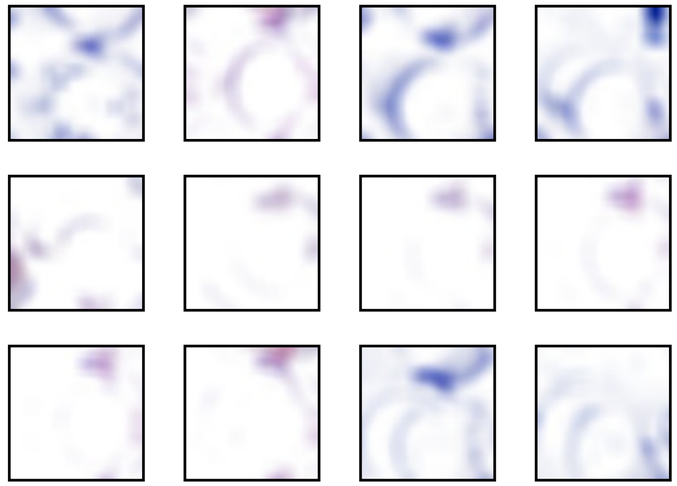
Machine Learning greatly helps!

Training data sets for ML

Positive



Negative

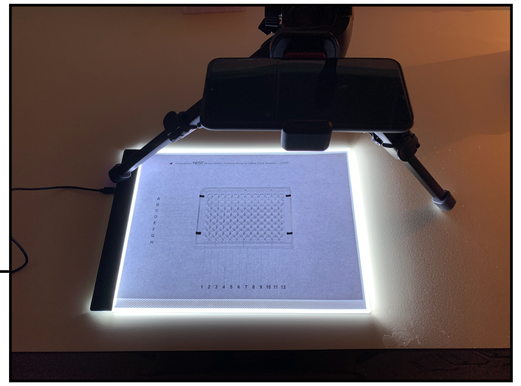


Programming environment and ML libraries

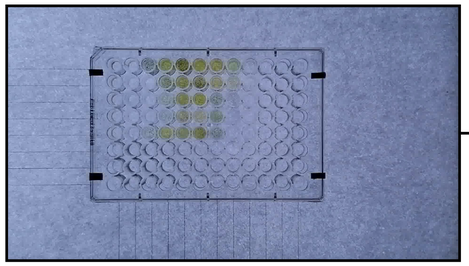
RStudio

Keras

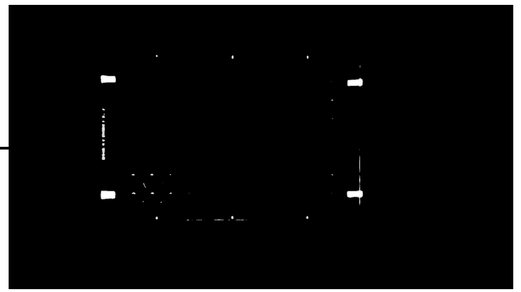
The algorithm of CAICR



movie recorded by cellular phone



transform to still image every 1 s



identification of wells on plate

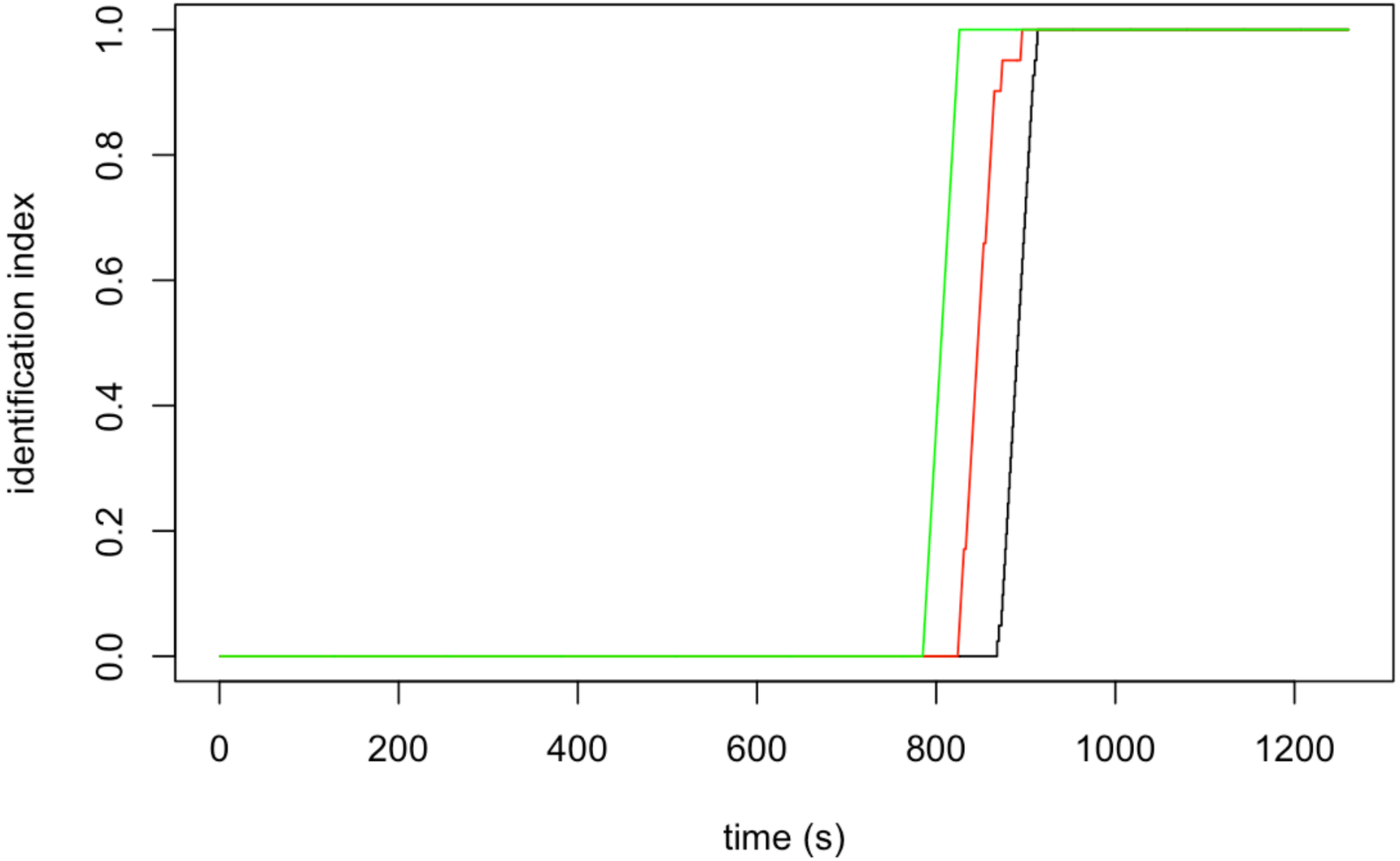
ML-based classification

assay output in CSV file

further analysis
or data interpretation

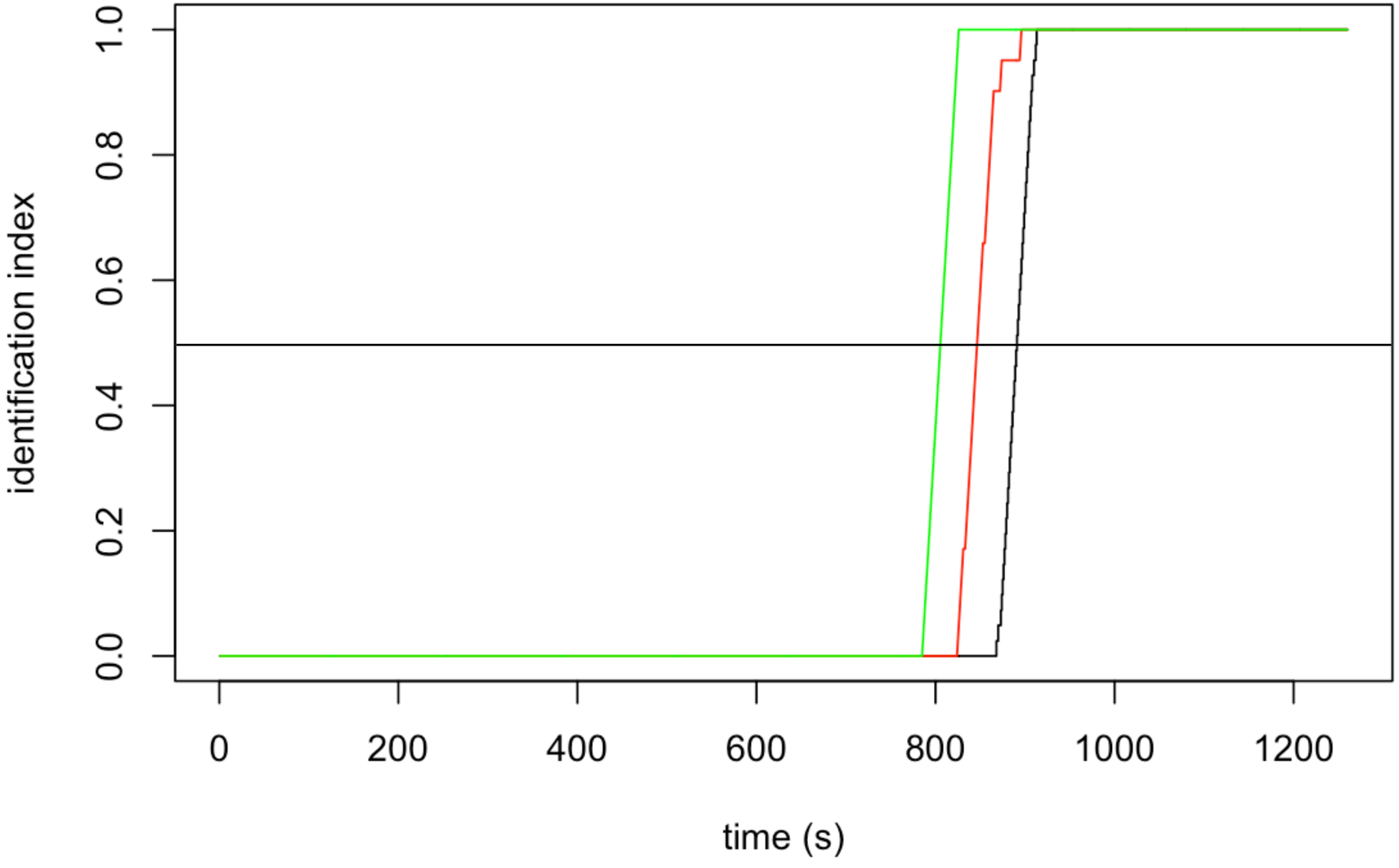
Simple moving average as an identification index

± 20 s (can be adjusted if needed)



Identification index threshold

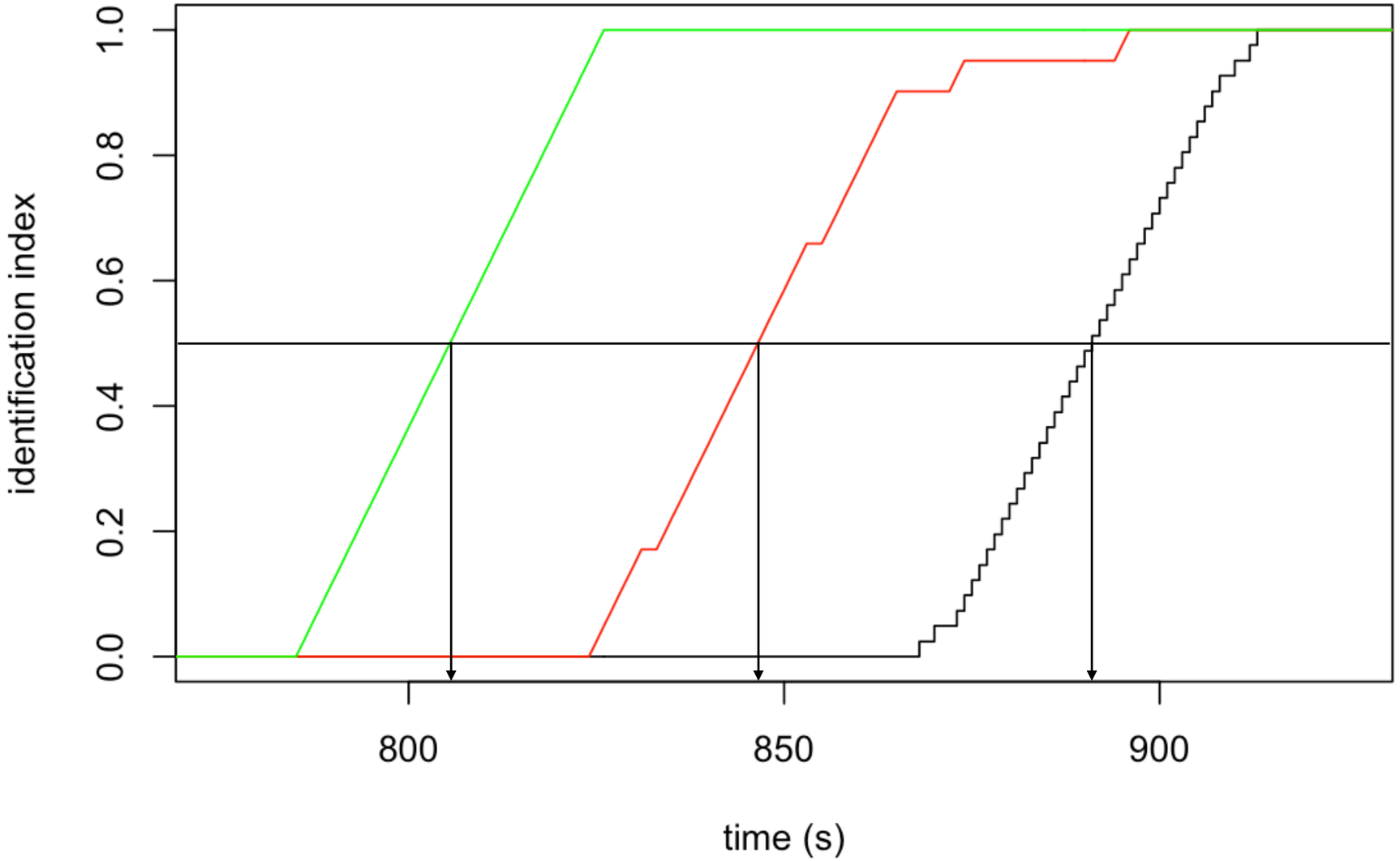
resembles Ct (cycle threshold) in qPCR



iit = 0.5

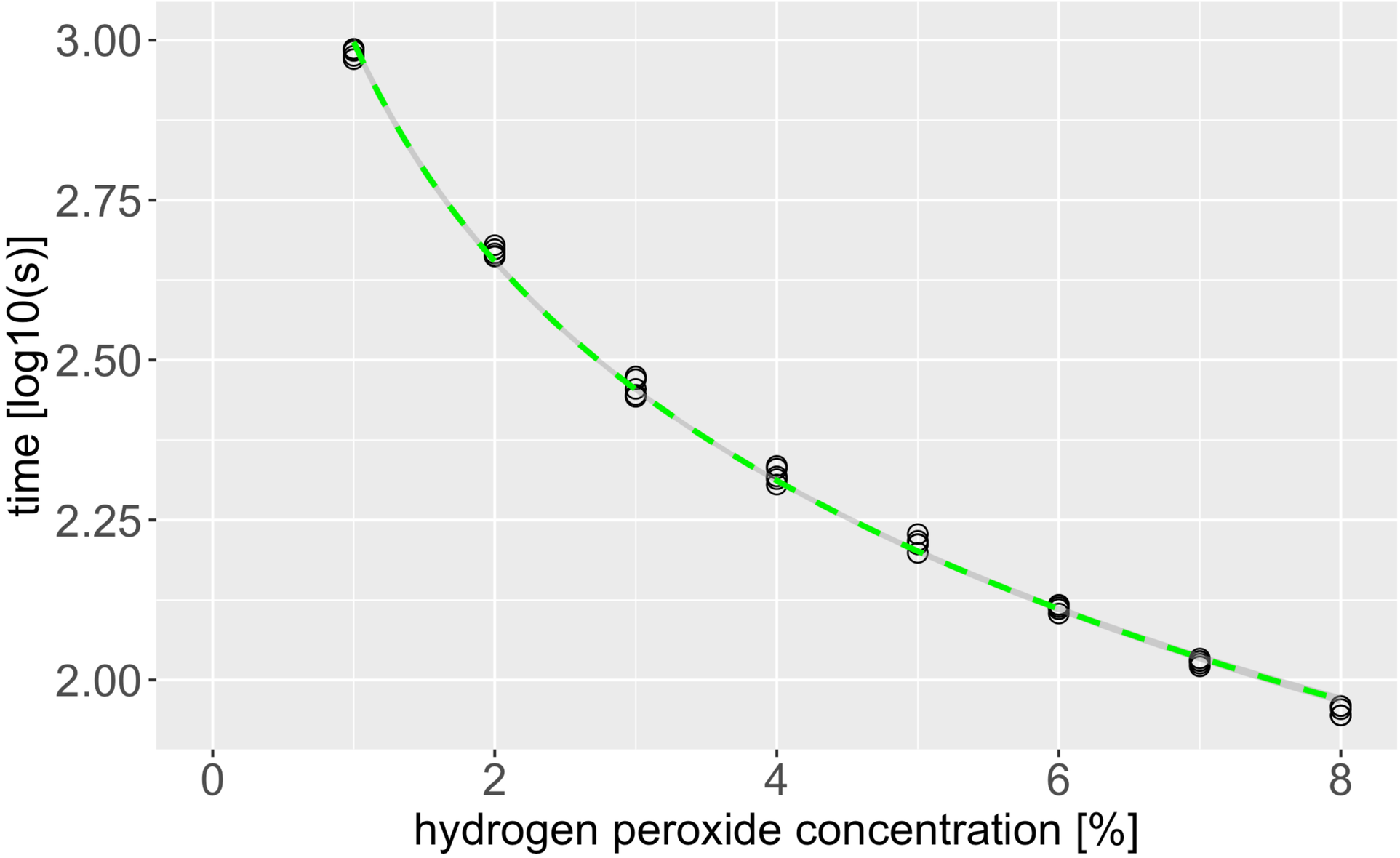
Non-biased automatic identification

resembles Ct (cycle threshold) in qPCR



iit = 0.5

CAICR really works!



Intra-Assay Coefficient of Variability

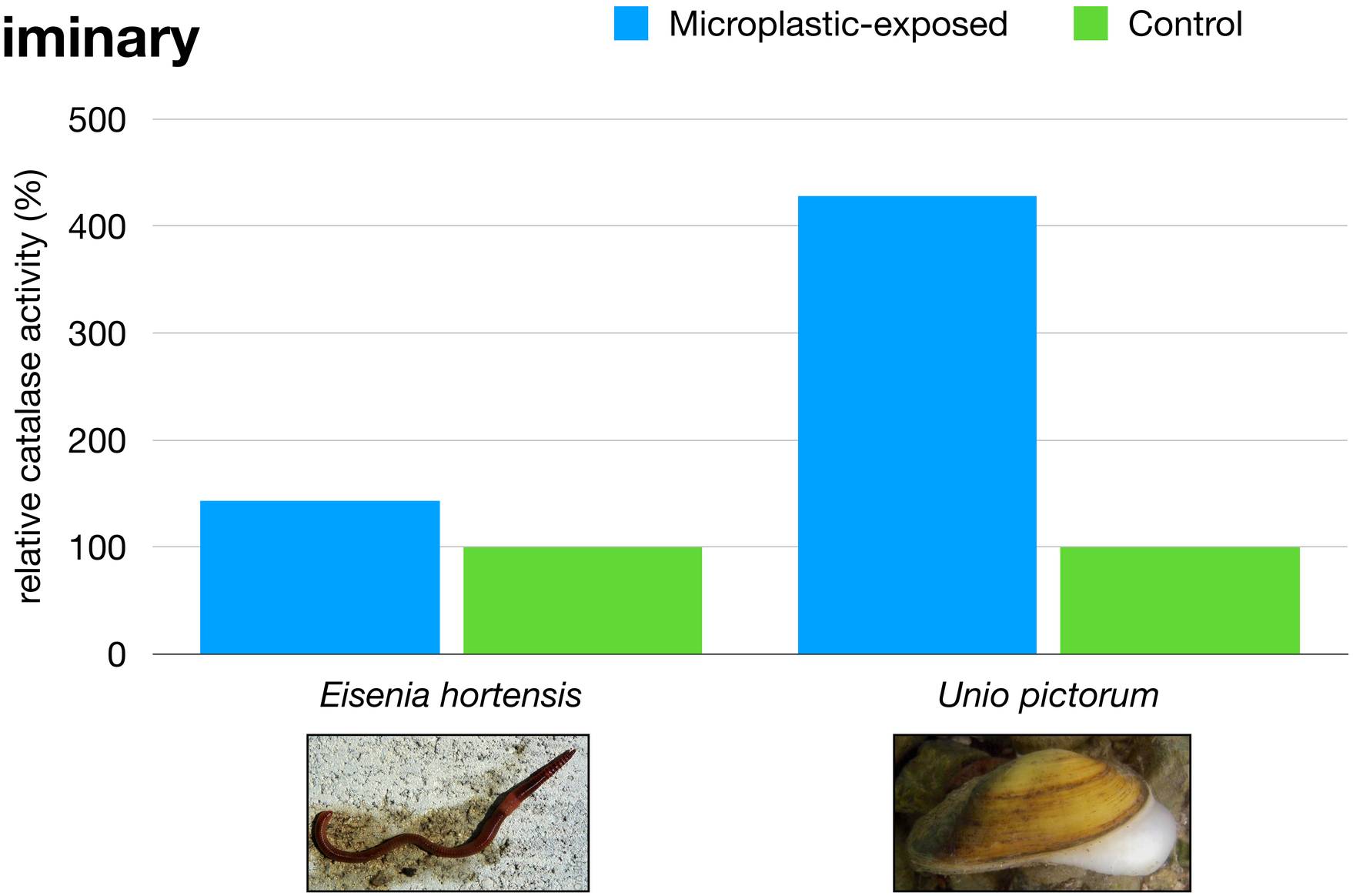
9.4%

Inter-Assay Coefficients of Variability

3.4%

CAICR in science

still preliminary



Conclusions

- Quantitative assay available for all
- Beautiful chemistry behind
- Cross-talk of chemistry and biology
- Programming skills in Python or R
- Glimpse of Machine Learning

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