MEASUREMENT OF CELLULASE ACTIVITY IN RABBIT CAECAL CONTENTS

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The core of the technique

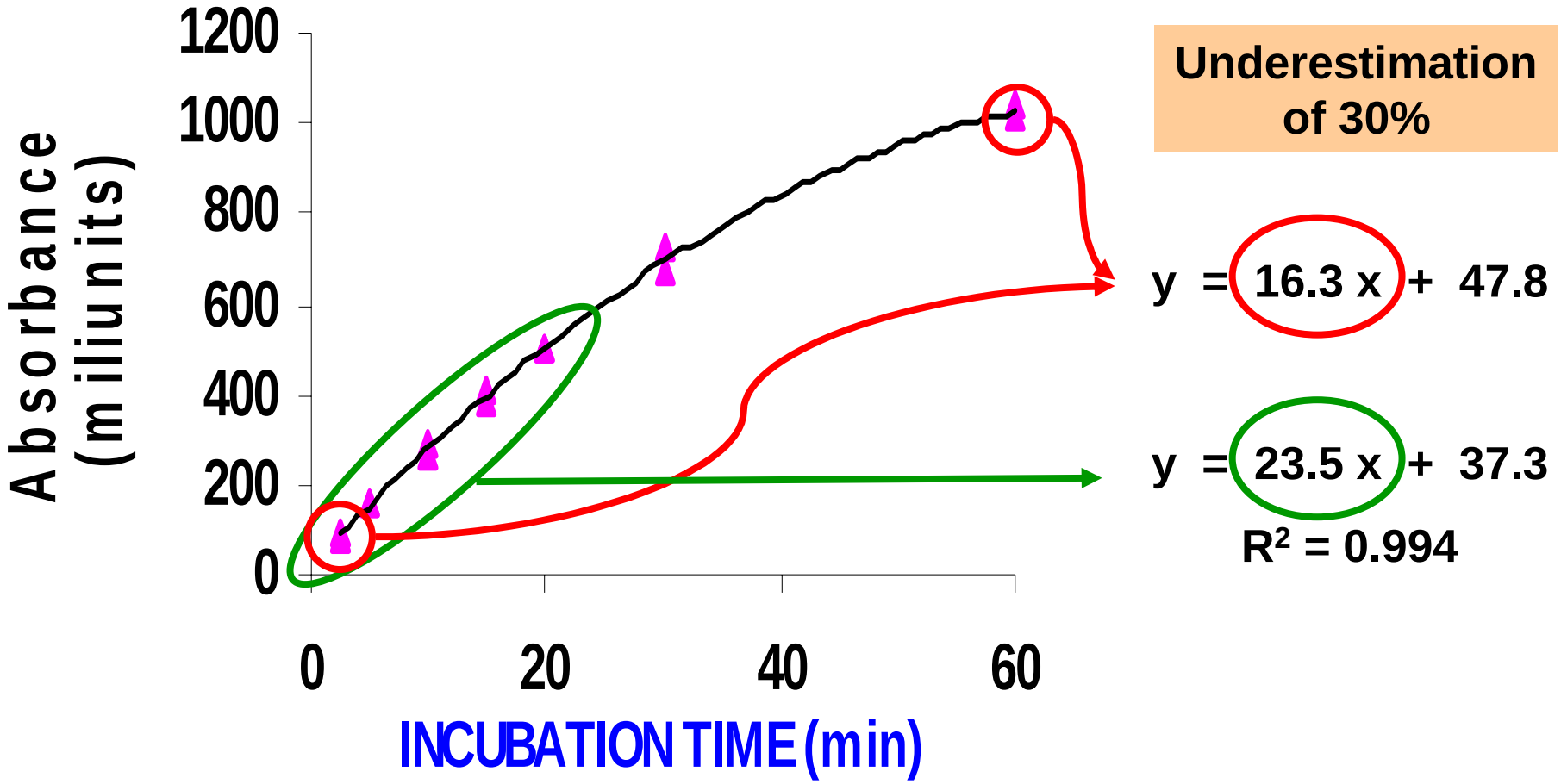
Hydrolysis of a substrate of carboximetilcellulose + **Determination of released reducing sugars**

by chromogenic reduction of the 2-hidroxi 3,5-dinitrobenzoic acid

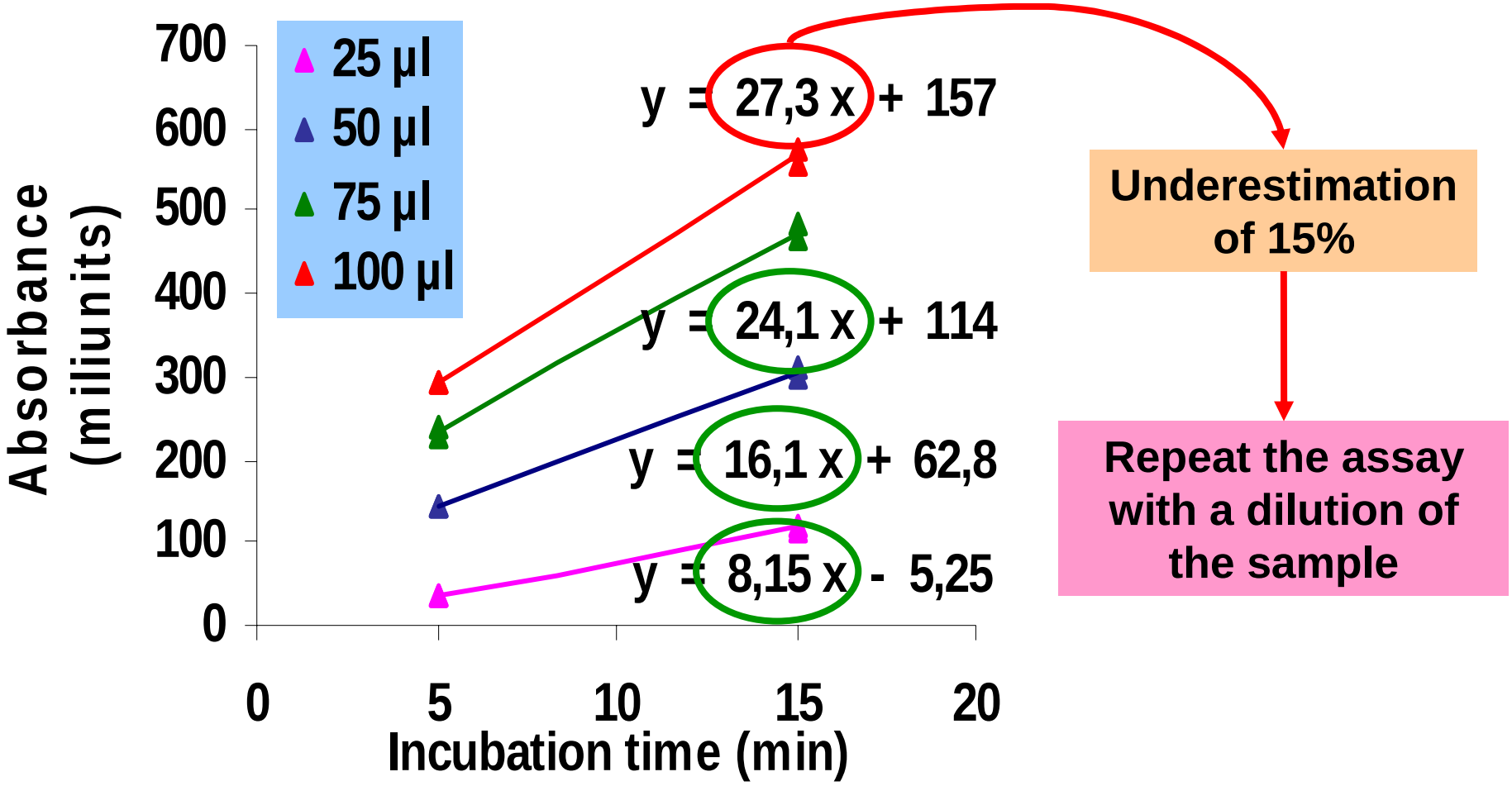
But the conditions of the assay must assure that the reaction rate

- **is constant along the incubation time**
- **is linearly dependent of the amount of enzyme present in the sample**

Constant reaction rate = linear relationship between the absorbance and the INCUBATION TIME



Linear relationship between the absorbance and the AMOUNT OF ENZYME



Our final procedure

1.5 ml of substrate (10 mg carboximetilcellulose/ml in citrate buffer 100 mM pH 6.0)

39 ºC

100 µl of sample

Incubate from 5 to 15 minutes

Stop reaction by addition of 1.5 ml of 2-hidroxi 3,5-dinitrobenzoic acid reagent

Heat in bath at 100 ºC during 5 minutes

Cool and read absorbance at 540 nm (repeat with a dilution of sample if increase of absorbance is higher than 240 miliunits)

Prepare blank assays as described but adding the sample after the addition of 2-hidroxi 3,5-dinitrobenzoic acid reagent

Convert the increments of absorbance in µmol of glucose by using a standard of 0 to 600 µg of glucose/ml