

EVALUATION OF LONG-TERM BIOLOGICAL ACTIVITY OF TRASTUZUMAB 15.0 mg/mL (HERCEPTIN®) BY AN AD HOC ELISA METHOD

Inmaculada Suárez González¹, Natalia Navas Iglesias², Antonio Salmerón García³, José Cabeza Barrera³, Luis Fermín Capitán Vallvey¹

¹ Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avda. Fuentenueva s/n, 18071, Spain.

² Department of Analytical Chemistry- Faculty of Sciences, Biomedical Research Institute ibs. Granada, University of Granada, Avda. Fuentenueva s/n, 18071, Spain.

³ Hospital Pharmacy Unit, Complejo Hospitalario Universitario de Granada, Biomedical Research Institute ibs. Granada, E-18012, Spain.

COMPLEJO HOSPITALARIO UNIVERSITARIO GRANADA

Servicio Andaluz de Salud CONSERJERÍA DE IGUALDAD, SALUD Y POLÍTICAS SOCIALES

BACKGROUND

Trastuzumab (TRZ) (Herceptin®) is a humanized monoclonal antibody IgG1 that **acts against** receptor 2 human epidermal growth factor (HER2). It is indicated in the **treatment of early and metastatic breast cancer and metastatic gastric cancer**.

PURPOSE AND OBJECTIVE

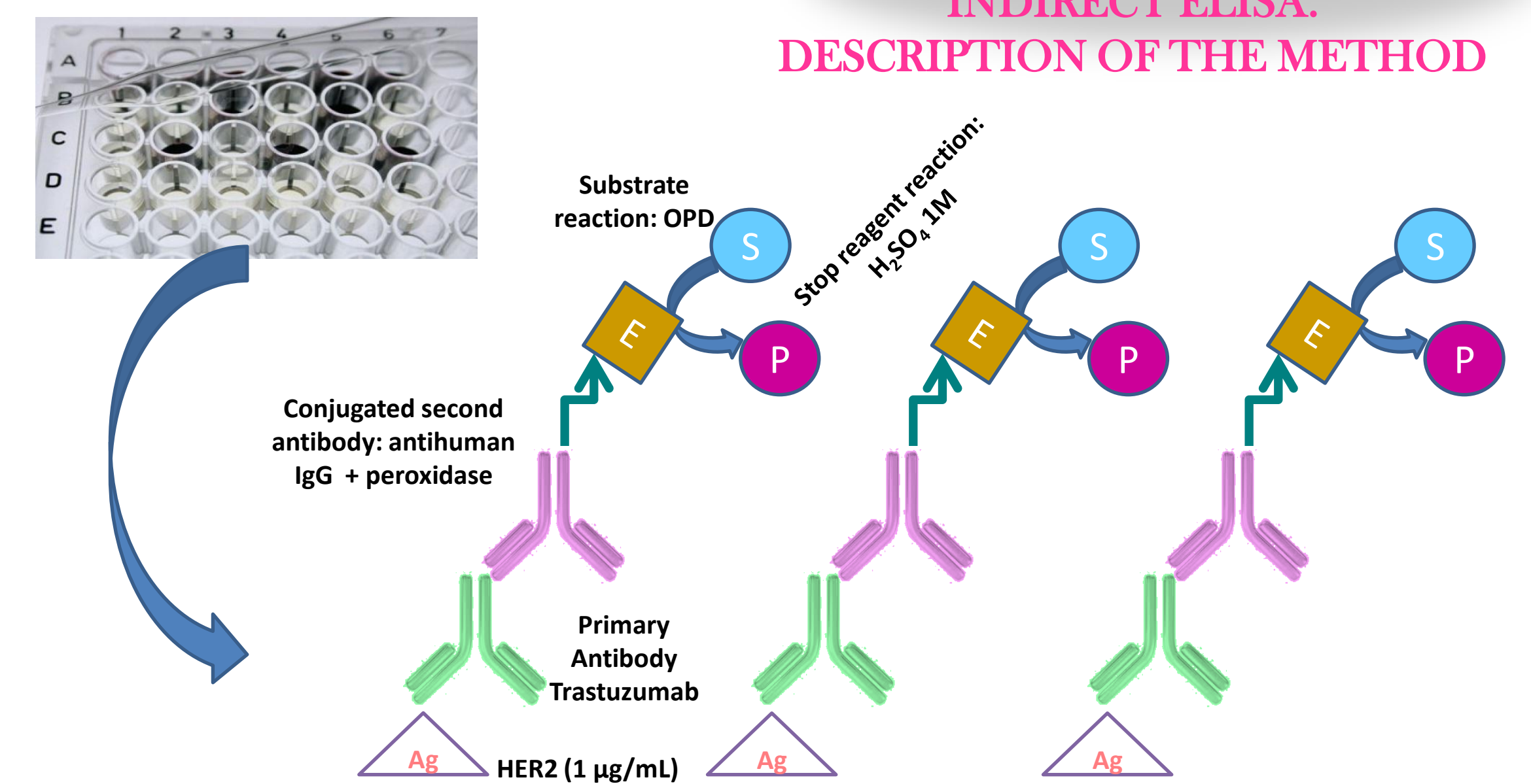
To evaluate the **post-biological activity** that remains in Herceptin® after opening single-use vials in long term study. It was also evaluated the remaining activity when exposing TRZ to different stress conditions.

EXPERIMENTAL



VALIDATION OF IMMUNOASSAY

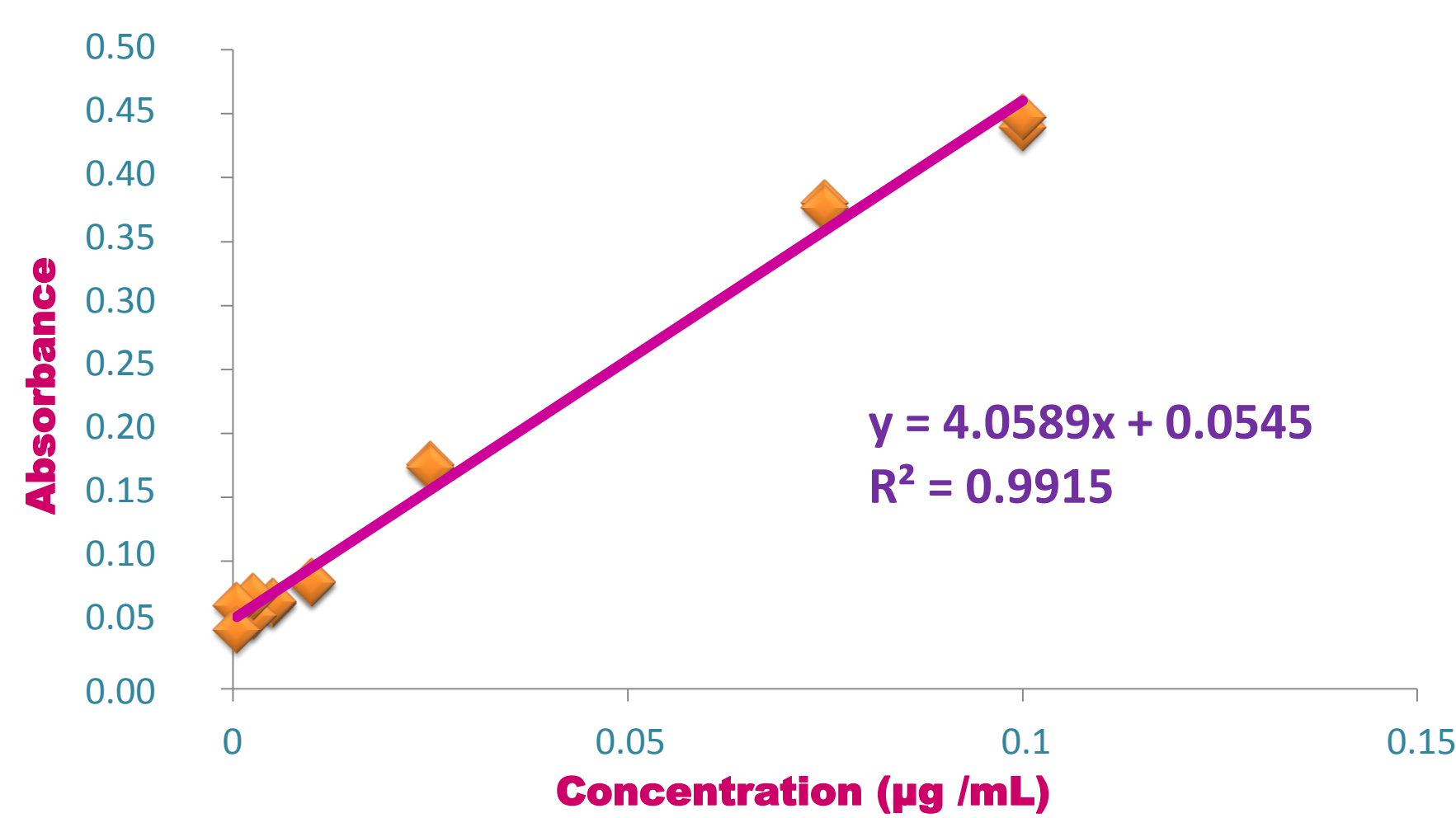
It was developed an *ad hoc* indirect non competitive ELISA based in the use of recombinant human HER2 to test **Biological Activity of Trastuzumab**.



INDIRECT ELISA. DESCRIPTION OF THE METHOD

The developed ELISA test has been validated in terms of calibration function, sensitivity as detection and quantification limits, accuracy (as % of recovery), and precision (as intraday and interday reproducibility % RSD).

CALIBRATION FUNCTION



SENSITIVITY

DETECTION LIMIT	31.8 ng/mL
QUANTITATION LIMIT	100.0 ng/mL
SENSING RANGE	100.0-500.0 ng/mL
DETECTION INTERVAL	31.8-100 ng/mL

ACCURACY

CONCENTRATION (µg/mL)	ABSORBANCE AVERAGE (450-620 nm)	% RECOVERY
0.4	0.6629	109.38 %
0.1	0.2635	105.95 %
0.01	0.1034	95.74 %

PRECISION

CONCENTRATION (µg/mL)	STANDARD DEVIATION	ABSORBANCE AVERAGE (450-620 nm)	COEFFICIENT OF VARIATION (% RSD)
REPEATABILITY			
0.4	0.0186	0.6629	2.81 %
0.1	0.0108	0.2895	3.74 %
0.01	0.0039	0.0859	4.63 %
REPRODUCIBILITY			
0.4	0.0680	0.6698	10.16 %
0.1	0.0430	0.3894	11.04 %
0.01	0.0027	0.0979	2.78 %

RESULTS

DRUG DEGRADATION STUDY

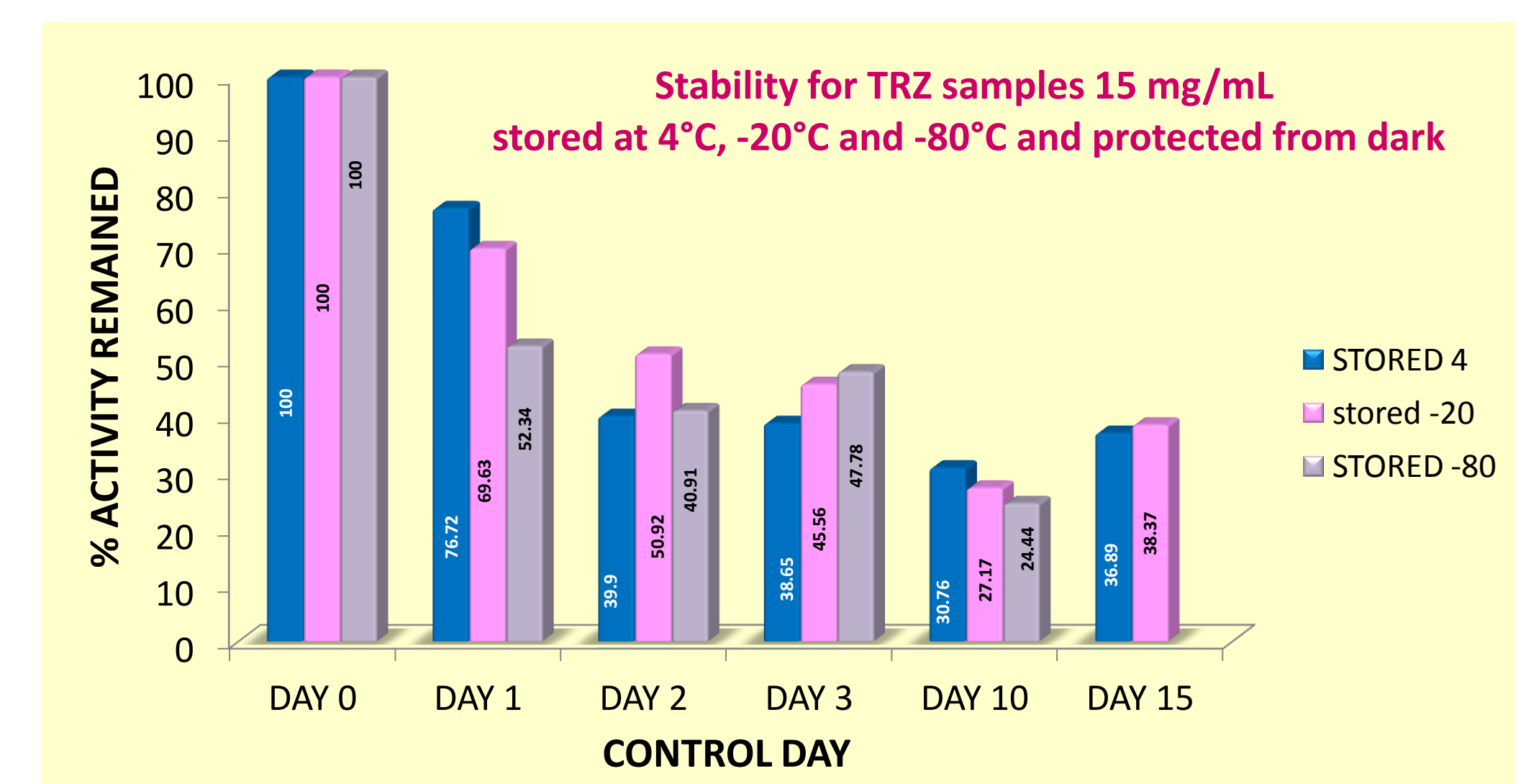
CONCENTRATION	15.0 mg/mL → Abs. Reference: 0.4306
STRESS CONDITIONS (24 h.)	
AVERAGE ABSORBANCE	
NaOH 0.1 M	0.2050
HCl 0.1 M	0.2410
H ₂ O ₂ 1% (v/v)	0.2360
H ₂ O ₂ 10% (v/v)	0.2305
NaCl 1 M	0.2320
50°C	0.2785
70°C	0.0763
UV 50°C 250 w/m	0.3823

Residual biological activity remained in all samples submitted to the stress except in samples heated at 70°C.

Stability Study

Surplus samples of Herceptin® from the daily use of the Hospital Pharmacy Unit were stored at 4°C, -20°C and -80°C protected from dark. Biological activity was tested up for 15 days.

The biological activity of Herceptin® decreased 25%, 30% and 47% the initial activity 24 hours after opening vials when stored at 4°C, -20°C and -80°C, respectively. The decrease was 50-60% after 2 days for the three storage conditions and it was maintained along the study (up to 15 days).



CROSS REACTIONS STUDY

TRZ ANTIGEN	BIOPHARMACEUTICAL	AVERAGE ABSORBANCE
HER2 1.0 µg/mL	TRZ 0.2 µg/mL	0.5495
HER2 1.0 µg/mL	RTX 0.2 µg/mL	0.0480
HER2 1.0 µg/mL	IFX 0.2 µg/mL	0.0360
HER2 1.0 µg/mL	BVZ 0.2 µg/mL	0.0497
HER2 1.0 µg/mL	CTX 0.2 µg/mL	0.0453

There were not cross reactions with the rest of biopharmaceuticals analyzed.

CONCLUSIONS

Herceptin® underwent a significant decrease of the biological activity when tested by ELISA after 24 hours of storage both refrigerated (4°C) and frozen (-20°C and -80°C). Nevertheless, these results will be further investigated by flow cytometry.

No conflicts of interest.

ACKNOWLEDGEMENTS

Financial support: P110/00201 (MICINN, Government of Spain)

