PP-026

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

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BACKGROUND

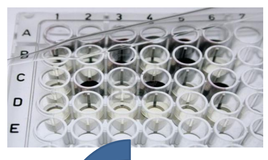
Trastuzumab (TRZ) (Herceptin®) is a humanized monoclonal antibody IgG1 that **acts against** receptor 2 human epidermal growth factor (HER2). It is indicated in the **treatment of early and metastatic breast cancer and metastatic gastric cancer**.

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

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

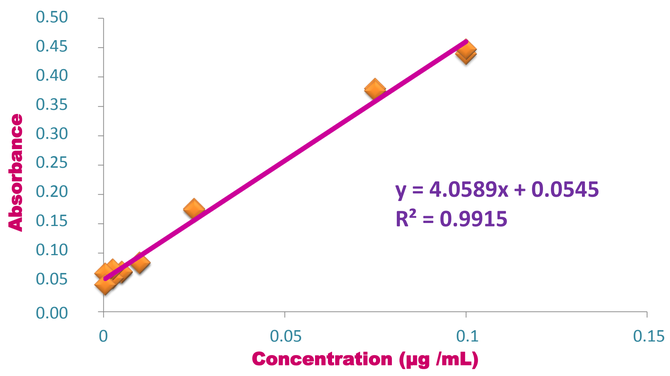


INDIRECT ELISA. DESCRIPTION OF THE METHOD

VALIDATION OF IMMUNOASSAY

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) n=8	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_2O_2 1% (v/v)	0.2360
H_2O_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.

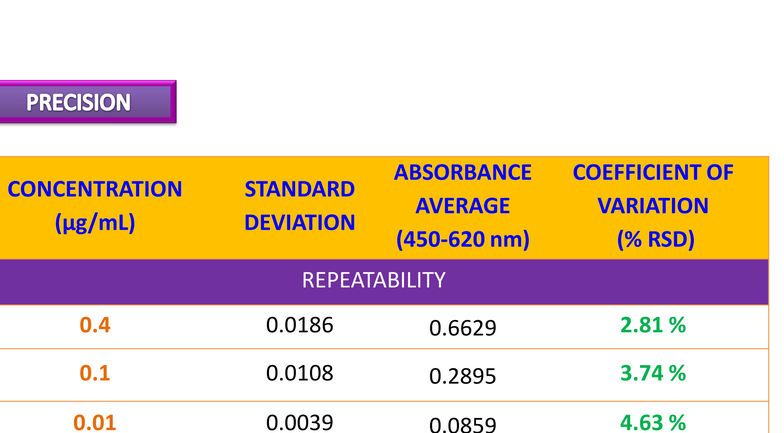
CROSS REACTIONS STUDY		
TRZ ANTIGEN	BIOPHARMACEUTICAL	AVERAGE ABSORBANCE
HER2 1.0 µg /mL	TRZ 0.2 µg /mL	0.5495
HER2 1.0 µg /mL	RTX 0.2 µg /mL	0.0480
HER2 1.0 µg /mL	IFX 0.2 µg / mL	0.0360
HER2 1.0 µg /mL	BVZ 0.2 µg / mL	0.0497
HER2 1.0 µg /mL	CTX 0.2 µg / mL	0.0453

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

Stabilty 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).



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

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