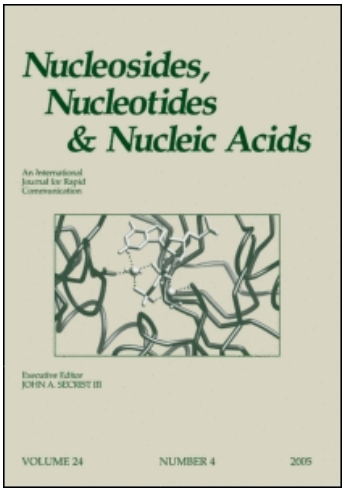

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Quantification of Lamivudine (3TC) by Competitive Immunoassay

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QUANTIFICATION OF LAMIVUDINE (3TC) BY COMPETITIVE IMMUNOASSAY

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ABSTRACT : Lamivudine or 3TC, the (-) eniantiomer of 2'-deoxy-3'-thiacytidine, is a prototype of a novel class of levogyre dideoxynucleosides analogues used in treatment of HIV and HBV infection. We describe a method corresponding to the first enzyme immunoassay for quantifying this antiviral drug. This technique use an enzyme conjugate that not require the use of radioactive labelling. In this study, anti-3TC antibodies were raised in rabbits by immunising with 3TC-HS-kelhoyle limpet hemocyanin (KLH) conjugate

We develop a method corresponding to a sensitive enzyme immunossay for quantifying the antiviral drug, 3TC, mainly used in anti-HIV or anti-HBV therapy. This method would allow an easy monitoring of kinetic parameters for patients at different stages of HIV disease or undergoing different therapy. This procedure may also extended to the measurement intracellular of this active phosphorylated metabolite of 3TC after previous separation followed by hydrolysis by alkaline phosphatase.

In this study, anti-3TC antibodies were raised in rabbits by immunising with 3TC-HS-kelhoyle limpet hemocyanin (KLH) conjugate. Conventional competitive immunoassays using these rabbit antisera as first antibodies and 3TC-HS-AChE conjugate as tracer were performed in 96-well microtiter plates coated with mouse monoclonal anti-rabbit IgG antibodies as specific second antibodies (FIG. 1).

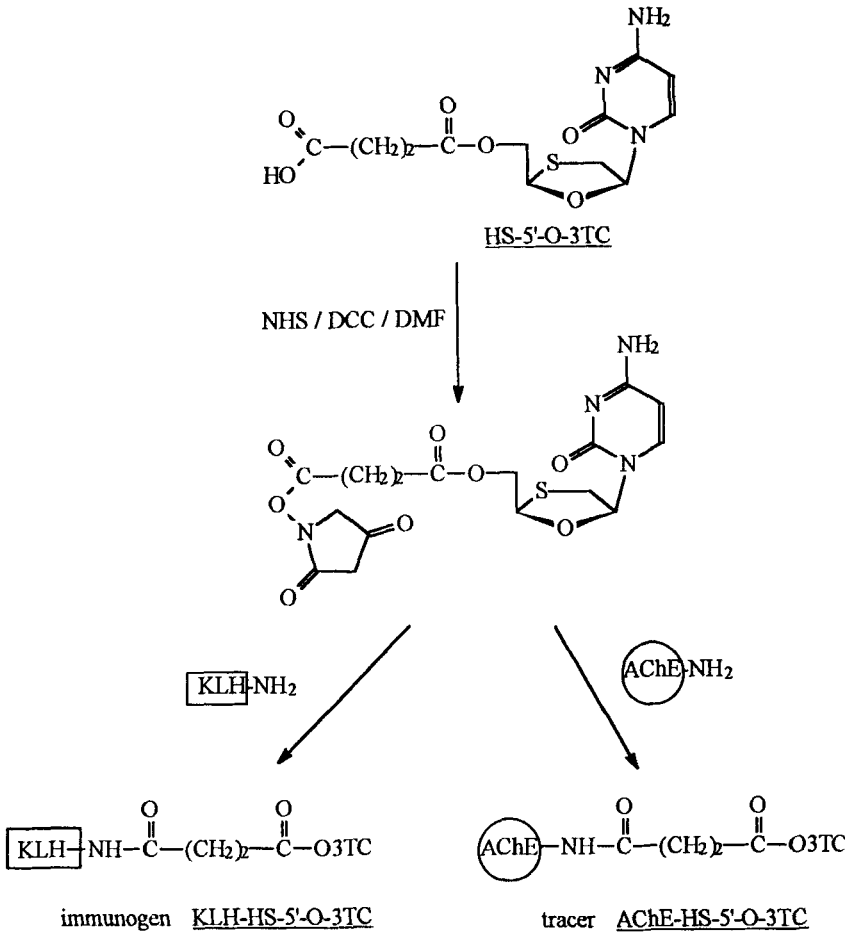

FIG. 1 : Synthesis of immunogen and tracer

Anti-3TC antibodies were detectable at 1/5000 and 1/25000 dilution at the first bleeding. After the final booster, the last bleeding dilutions used were 1/500000. Thus demonstrating the immunogenic potency of the 3TC-HS-KLH immunogen.

This assay proved to be sensitive (B/Bo 50% : 260 pg/ml) with a MDC close to 50 pg/ml. The precision of the assay is good with a coefficient of variation less than 10 % in the 156-5000 pg/ml range (FIG. 2).

All nucleoside and nucleotide tested exhibit a poor cross-reactivity (less of 1.6 %). The main metabolite of 3TC i.e. sulfoxy-3TC demonstrates a poor cross reactivity, close to 1.5 % (Table I).

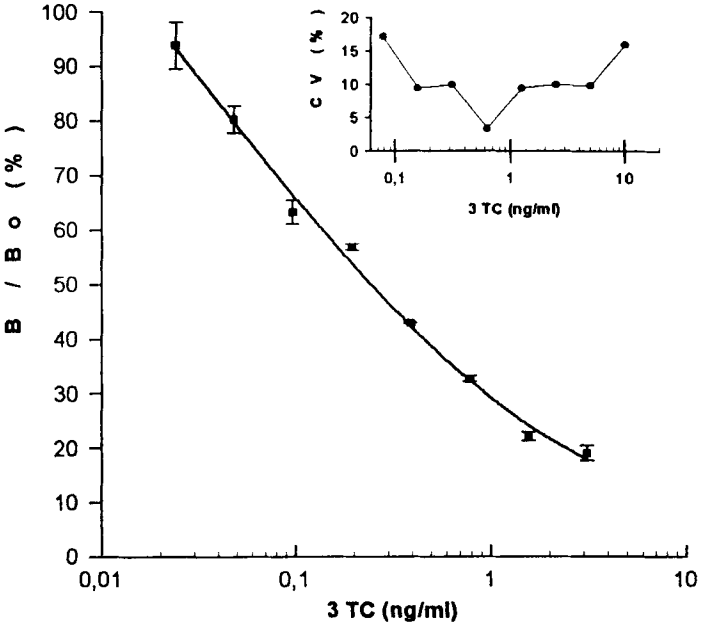

FIG. 2 : Typical calibration curve of 3TC.
The results are expressed in terms of B/Bo as a function of the logarithm of the dose of 3TC. All measurements for standards were made in duplicate. Each bar represents one standard deviation.
Insert : Precision profile for the present assay using data collected for the standard curve with 8 replicates for each dose of 3TC in 156-5000 pg/ml. The precision of the assay is expressed as cœfficient of variation CV vs. the dose (logarithmic scale).

TABLE 1 : Cross-reactivity values for 3TC analogues and nucleosides.
Cross-reactivity (CR) : [(dose of 3TC B/Bo 50 % / dose of analogue B/Bo 50 %) x 100].

Compound	Cross-reactivity
Lamivudine or 3TC	100
3TC-HS	100
Thymidine*	<0.1
AZT*	<0.1
ddI*	<0.1
ddC	0.15
Cytidine*	<0.2
3TC-MP	0.5
3TC-TP*	<0.3
sulfoxy-3TC	1.5

*Estimated values because the highest concentration used for cross-reactivity analysis did not cause any detectable displacement.

This test is sensitive, detecting as little as 100 pg/ml under optimal conditions. This threshold is far better than that obtained by classical HPLC method. This assay was successfully applied to the determination of 3TC levels in human plasma HIV-infected patients.

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