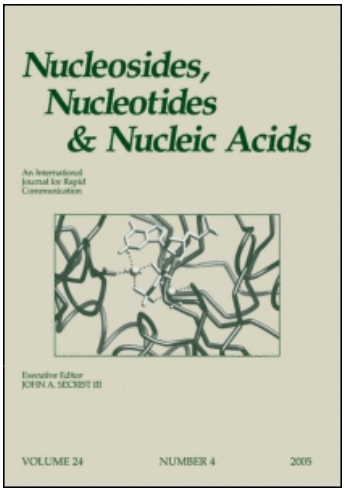

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THE USE OF (OLIGONUCLEOTIDE-MALEIC ANHYDRIDE COPOLYMER) CONJUGATES IN NUCLEIC ACID DIAGNOSTIC ASSAYS: EFFECT OF THE NUMBER OF OLIGONUCLEOTIDES PER POLYMER ON TEST SENSITIVITY IMPROVEMENT

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ABSTRACT

(Polymer-oligonucleotide) conjugates were obtained via direct ON synthesis from the poly(ethylene-*alt*-maleic anhydride: PEMA) grafted onto glass bead surfaces. The effect of the ON number per polymer chain on binding was evaluated through Tm experiments. Results were correlated with ELOSA (Enzyme Linked Oligosorbent Assay) tests run with conjugates in the capture step.

In the medical diagnostics field, a sandwich DNA hybridization test typically occurs in two steps. The DNA target is first immobilized onto a solid support before being detected, in a second step, by a specific probe that will subsequently provide a colorimetric signal. Specifically immobilizing the DNA target onto a support means being able to extract it from a biological fluid. For this purpose, the surface of the solid support must be modified by the immobilization of short oligonucleotides (ON). These nucleic acid probes can specifically bind to the DNA target, allowing its capture from the medium. To enhance the sensitivity of such a test, polymers like linear Poly (maleic anhydride-methyl vinyl ether) (1), Polyacrylamide gel (2,3) or Polyamine dendrimer (4) proved to be useful in the capture step. Indeed,

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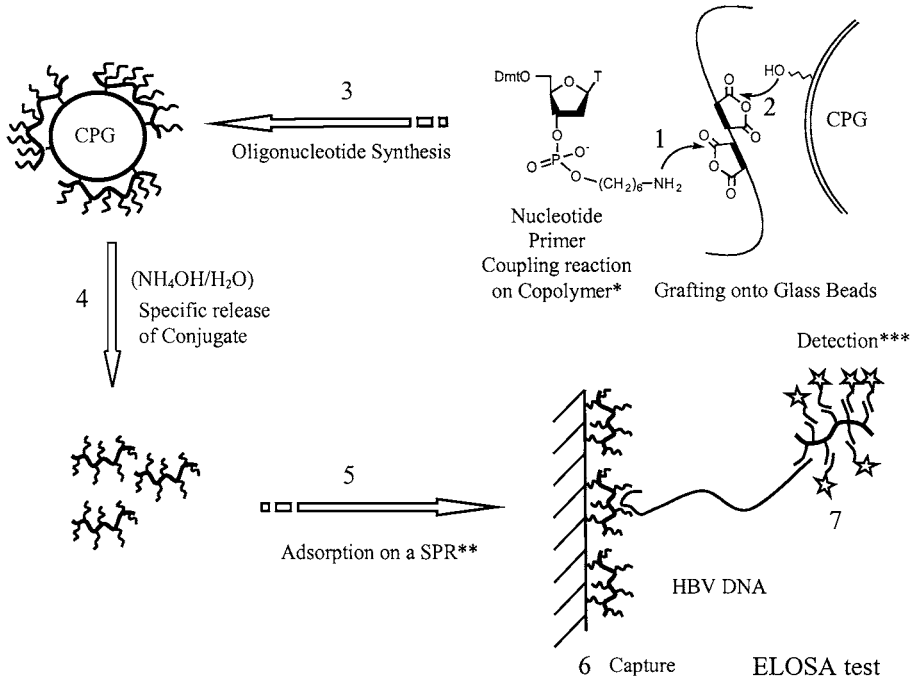
the presence of a polymer layer at the interface of the solid support increases the accessibility of probes which are covalently bound to the polymer. Consequently, the probes can bind more efficiently to the DNA target. Furthermore, as a general rule, polymers are hydrophilic in order to reduce non-specific adsorption on the surface.

In this study, we have evaluated the role of various conjugates (oligonucleotide-copolymer) in the capture step of the Hepatitis B Virus (HBV) diagnostic test, run on a bioMérieux VIDAS immunoassay instrument. Procedures for synthesizing conjugates and running the test are described in Figure 1. Conjugate synthesis was achieved following a strategy developed in the laboratory (5). The first step consisted of coupling a nucleotide (starter of ON synthesis) to the PEMA before grafting the resulting reactive polymer onto a Controlled Pore Glass (CPG) support, in a second step. The third step aimed to directly synthesize oligonucleotides from the polymer. Then conjugates were released in solution by ammonia treatment. The diagnostic assay was run as follows : conjugates were first adsorbed onto the surface of a conical Solid Phase Receptacle (SPR) from the VIDAS instrument (step 5), then a HBV DNA target (2339 bp) solution at 10^{10} copies/ml was introduced into



* Poly(ethylene-*alt*-maleic anhydride) ($\overline{Mn}$: 27000g/mol)
** Solid Phase Receptacle of VIDAS Instrument
*** Detection conjugate + enzyme labeled probes

Figure 1. Principle of conjugate syntheses and their use in a ELOSA sandwich test.

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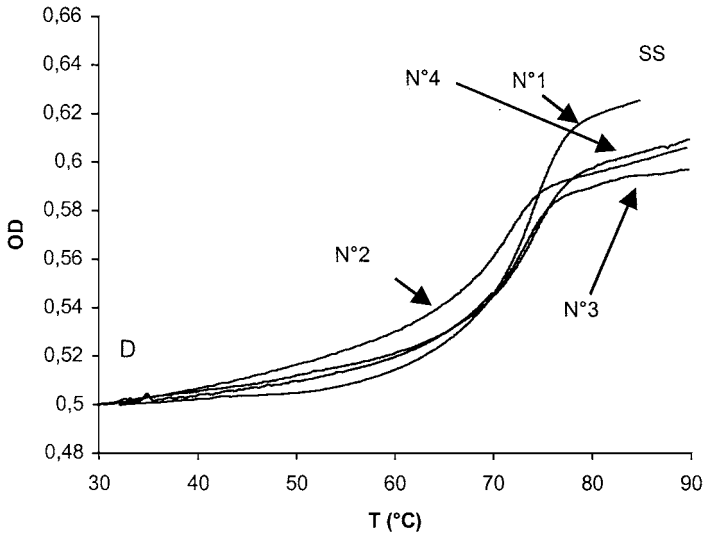
the SPR (step 6). At the detection step, a conjugate (polymer-ON) specifically hybridized to the target and a probe labeled by an enzyme hybridized to the detection conjugate (step 7). Classically, the polymer used for the detection conjugate was the Poly (N-vinylpyrrolidone N-acryloxysuccinimide) (6). The resulting colorimetric signal enabled the efficiency of the HBV DNA capture to be evaluated.

By modifying the concentration of the nucleotide primer at the first step of the synthesis, three different conjugates were prepared. Each of them contained a different number of ON on the polymer chain (19, 92 and 109 oligonucleotides per PEMA respectively). The sequence corresponded to the capture probe of the test (Fig. 2) with five additional thymidines at the 3′ extremity in order to distance the capture sequence from the polymer chain. Melting Temperature (Tm) experiments



	Tm (°C)	Hyperchromicity*
N°1 : ON Capture**	73.9°C	100%
N°2 : 19 ONs/PEMA	71.5°C	57%
N°3 : 92 ONs/PEMA	73.4°C	68%
N°4 : 109 ONs/PEMA	74.1°C	72%

* $\frac{\text{Abs (260nm) Single Strands} - \text{Abs (260nm) Duplex}}{\text{Abs (260nm) Duplex}}$
in percentage related to N°1 reference hyperchromicity considered to 100%.
** 5' TCA ATC TCG GGA ATC TCA ATG TTA G 3'

Figure 2. Melting temperature experiments with conjugates.

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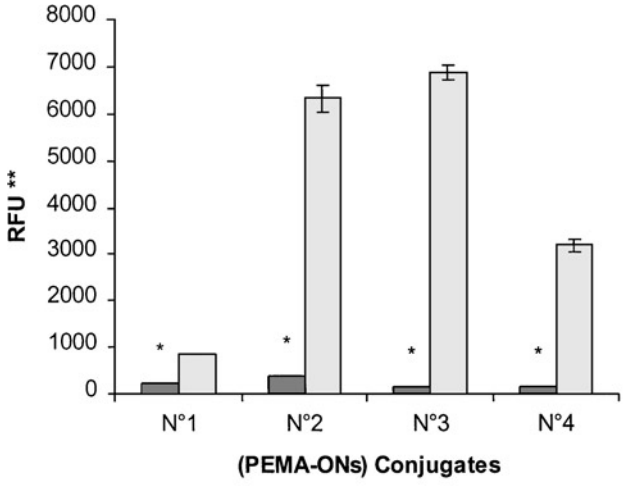
were run with these conjugates. Curves were compared to that of the capture oligonucleotide with its complementary sequence. For each experiment run with conjugates, a transition was observed that confirmed a good binding to complementary sequence. Melting Temperatures are summarized in Figure 2. The Tm of the two conjugates highly loaded with ONs (73.4°C for N°3 and 74.1°C for N°4) were close to that of the standard (73.9°C for ON Capture). However, the Tm of the conjugate with 19 ONs per chain was lower (71.5°C). The same trend was observed when we calculated the percentage of hyperchromicity related to that of the standard. Values for N°3 and N°4 were close. But the transition amplitude was lower with conjugate N°2. Although we can hardly conclude on the efficiency of the hybridization of ONs bound to the polymer in comparison with the standard system, we can nevertheless notice that ON binding to conjugate N°2 was less efficient. This could result from a different conformation of this conjugate in solution limiting oligonucleotide accessibility and reducing hybridization.

These conjugates have been tested in the capture step of a sandwich diagnostic assay as described in Figure 1. Results are reported in Figure 3. Firstly, the three conjugates led to increased test sensitivity in comparison with the capture sequence used as a control. Nevertheless, the conjugate giving the lowest signal was that bearing the higher oligonucleotide functionalization (N°4). Such a decrease could be explained by steric hindrance phenomena at the surface of the SPR after adsorption of the conjugate. In the latter case, capture probe accessibility was probably reduced. On the other hand, conjugate N°2 gave the same RFU signal as N°3. However, the background signal increased, indicating that this conjugate was not the best candidate. The signal/noise ratio was maximum for N°3 conjugate.



*Background : experiment run without HBV DNA target
** RFU : Relative Fluorescence Unit

Figure 3. HBV DNA diagnostic test run with conjugates.

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In conclusion, binding studies with complementary sequences have confirmed the efficiency of the duplex formation, more particularly for conjugates N°3 and N°4. On the other hand, VIDAS experiments run with the conjugates in the capture phase revealed a decrease in target capture efficiency for the conjugate with the highest number of oligonucleotides per polymer chain. After adsorption of such a conjugate, the observed reduction in capture probe accessibility may arise from steric hindrance phenomena or compactness of the conjugate.

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