A NUCLEIC ACID-BASED RAPID DIAGNOSTIC TEST

FOR DETECTION OF WEST NILE VIRUS (WNV)

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INTRODUCTION

Rapid and accurate pathogen detection plays an important role in the containment of local disease outbreaks, which is essential to prevent health emergencies. As a part of the MOBILISE project aiming at establishing a mobile laboratory responding to disease outbreaks in areas lacking stationary laboratory infrastructure, we are developing a rapid diagnostic workflow for diagnosis of West Nile Virus (WNV) infection, which is becoming a public health concern in Europe due to climate change (1). The presented procedure involves lateral flow devices (LFDs) for rapid detection of the viral nucleic acid.

RECOMBINASE POLYMERASE AMPLIFICATION (RPA)

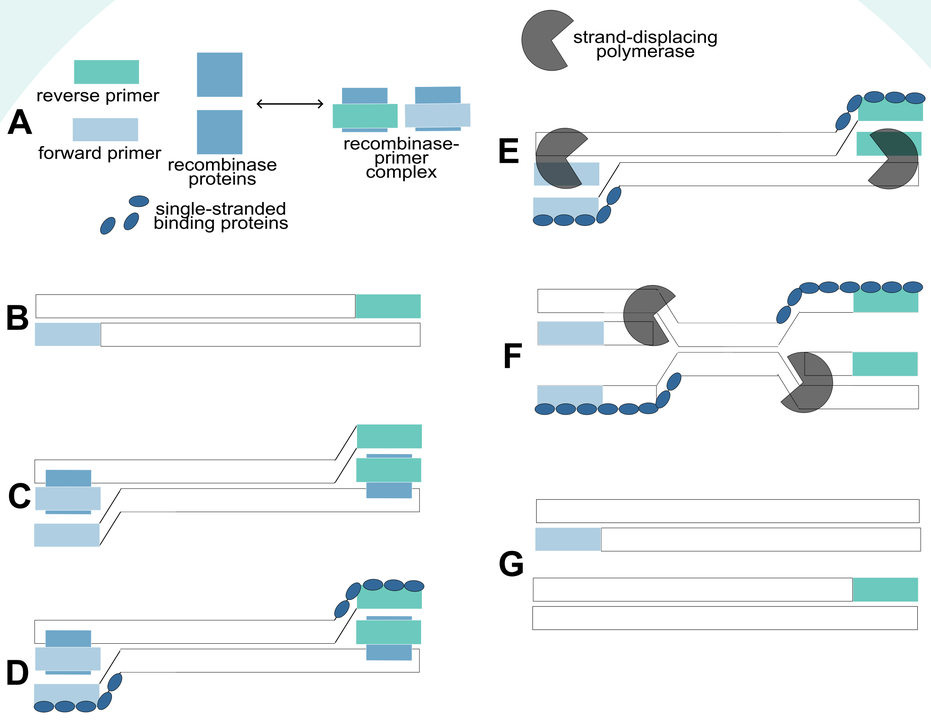

Figure 1 A-G (modified from (2), open access)**:** Principle of Recombinase Polymerase Amplification (RPA): Recombinase proteins form a complex with the forward and reverse primers (A) and direct them to their respective binding sites in the double-stranded template (B/C). Single-stranded binding proteins stabilize the resulting single-stranded parts of the template (D). A strand displacing polymerase then elongates the forward and reverse primers (E/F), resulting in the formation of double-stranded amplicons (G). The entire process takes place isothermally between 37 and 42 °C.

CONCEPT OF LATERAL FLOW DETECTION

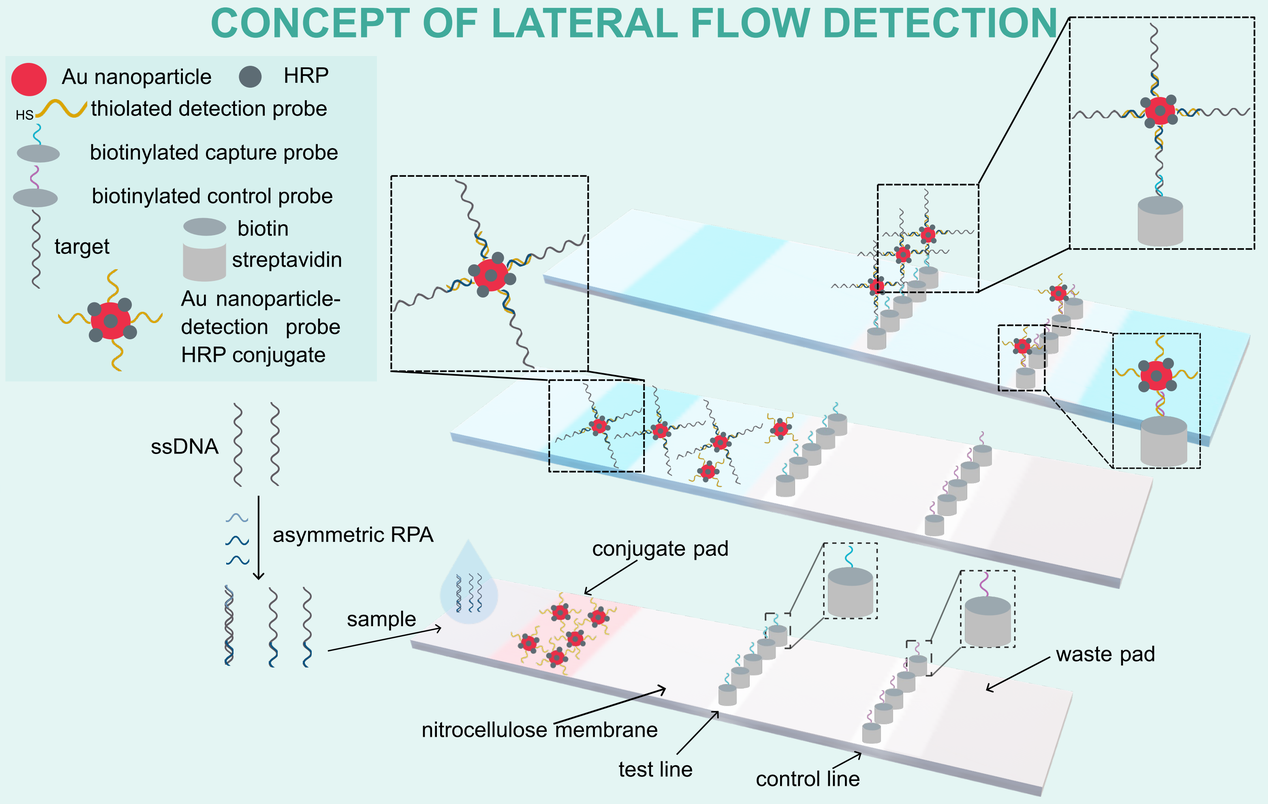

Figure 2: Concept of the developed diagnostic procedure targeting WNV nucleic acid: WNV ssDNA is amplified via asymmetric RPA, resulting in mainly single-stranded amplicons. The amplicons are then applied to the sample pad of the LFDs. Due to capillary forces, the amplicons travel to the conjugate pad, where they are bound by detection probes immobilized on the AuNP-DNA-HRP conjugate. When these assemblies reach the test line, they are captured by immobilized DNA probes, leading to an accumulation of gold nanoparticles and therefore a visible red band. Excess AuNP-DNA-HRP conjugate is bound by the control probes immobilized on the control line.

WITHOUT PREAMPLIFICATION

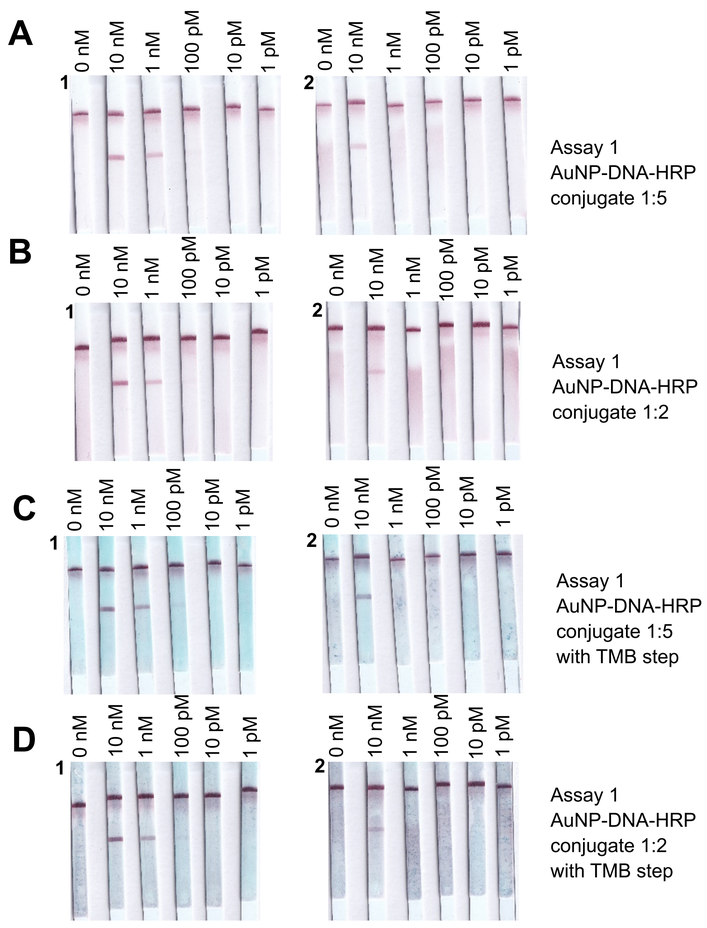

Figure 3 A-D: Sensitivity of the developed lateral flow devices for WNV ssDNA. Indicated concentrations of WNV ssDNA in running buffer (4x saline sodium citrate buffer with (**A/B/C/D 1**) or without 4% BSA (**A/B/C/D 2**) were applied directly to the LFDs. AuNP-DNA-HRP conjugate was diluted 1:2 or 1:5 after synthesis. Application of 3,3',5,5'-Tetramethylbenzidine (TMB) to the LFDs for 15 minutes served to enhance the signal on the test and control lines as TMB is converted to a blue product by the HRP on the AuNP-DNA-HRP conjugate. Prior to signal enhancement, 1 nM (~6 x 10^8 copies/µl) of WNV ssDNA can be detected when using 4% BSA in the running buffer (**A/B 1**). Sensitivity is improved by one order of magnitude (100 pM/ ~6 x 10^7 copies/ µl) after application of TMB (**C/D 1)**.

RPA PREAMPLIFICATION

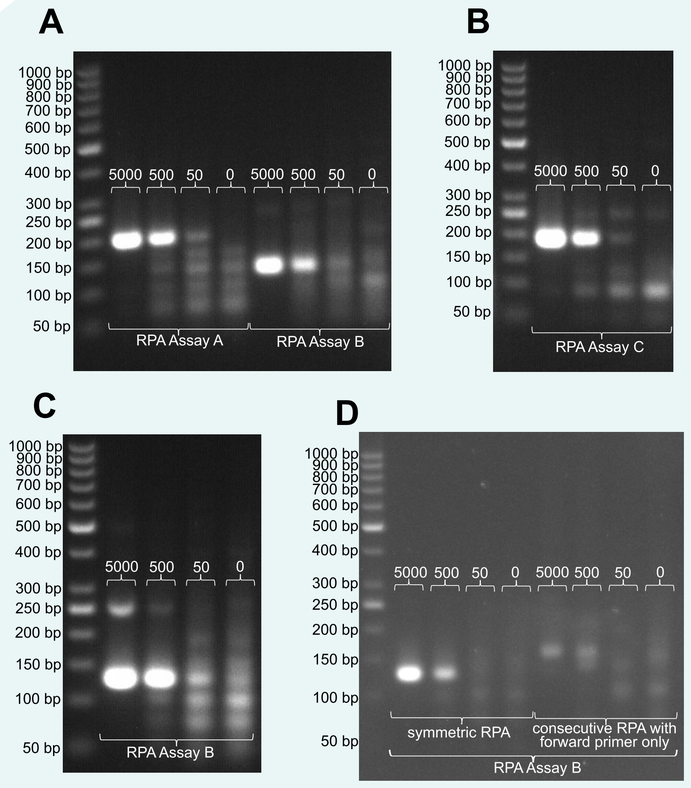

Figure 4 A-D: Three different RPA assays were designed for WNV ssDNA amplification and analyzed on agarose gels (**A/B**, forward and reverse primers in a 1:1 ratio, RPA at 39°C for 25 min). Overall, all assays showed relatively comparable band intensities and faint bands for 50 copies/reaction. Assay A showed more bands for unwanted by-products than assays B and C. The LFD only detects amplicon DNA in its single-stranded form. Thus, to generate ssDNA in RPA, an asymmetric RPA was performed using assay B (which showed best performance in asymmetric RPA) with a ratio of forward : reverse primer of 2 : 1, and the absolute primer concentrations were doubled (**C**). As an alternative approach, symmetric RPA was carried out at 39°C for 25 minutes (**D**) and a fraction of the resulting amplicons was used as templates for a second RPA reaction with forward primer only (39°C, 25 minutes, **D**). While the asymmetric RPA in **C** and the symmetric RPA step in **D** show bands for dsDNA amplicons, the second step shows no bands for the double-stranded amplicons, since the products in this step are single-stranded only. Products from both approaches were applied to LFDs for detection (see **Figure 5**).

LFD DETECTION

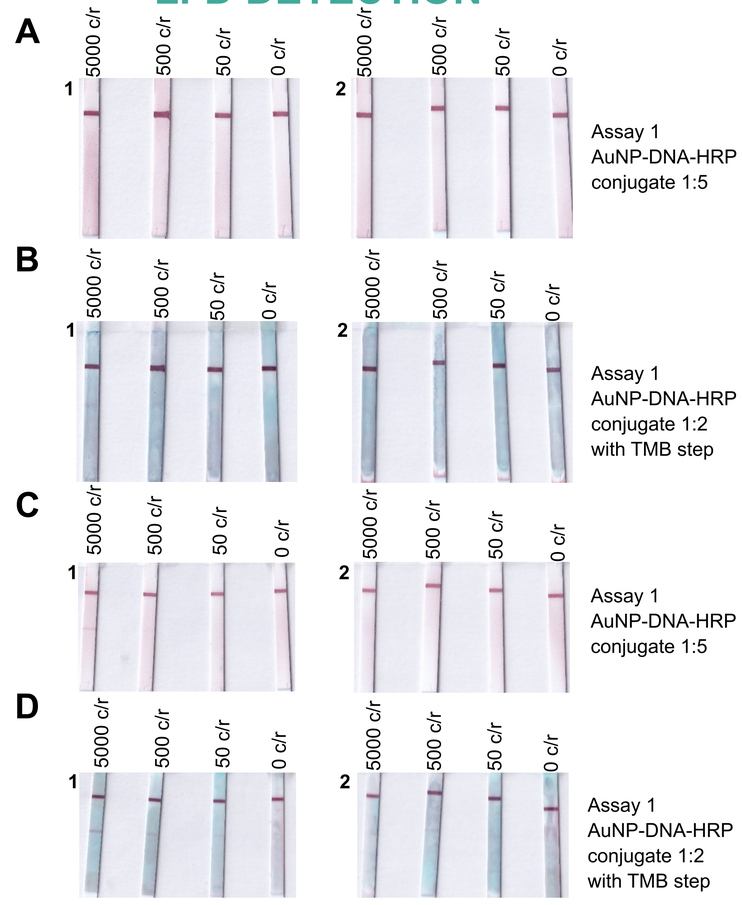

Figure 5 A-D: Detection of RPA amplicons with the developed LFDs. RPA was performed asymmetrically with a primer ratio of 2:1 at 39°C for 25 minutes (**A** (before addition of TMB) and **B** (after addition of TMB)) in one step or symmetrically (primer ratio 1:1) followed by a second step where only one primer was added (both at 39°C for 25 minutes, **C** (before TMB) and **D** (after TMB)). When applying one-step asymmetric RPA products to the LFDs, a faint signal is obtained at the test line for 5000 copies/reaction (c/r). In contrast, when amplicons from the two-step RPA procedure are added to the LFDs, positive signals are obtained for 5000 and 500 copies/reaction. Both workflows lead to a significant enhancement in sensitivity compard to the LFDs without RPA preamplification.

CONCLUSION & OUTLOOK

In conclusion, we have developed a workflow for detection of WNV ssDNA consisting of RPA preamplification combined with direct detection of the amplicons via nucleic acid-based LFDs. Sensitivities down to 500 copies/reaction were achieved within 60 minutes (with TMB step) or 45 minutes (without TMB step). It is possible to rapidly adapt our dignostic procedure to new virus variants by simply changing the sequence of the RPA primers and oligonucleotides immobilized on the LFD. Due to good sensitivity, fast time-to-result, ease of operation and straightforward adaptability to new emerging virus variants, this method offers good potential for application in mobile laboratories responding to virus outbreaks in remote areas, where rapid identification of infected individuals is indespensable to prohibit spreading of the disease.

(1) Farooq, Z.; Sjödin, H.; Semenza, J. C.; Tozan, Y.; Sewe, M. O.; Wallin, J.; Rocklöv, J. European projections of West Nile virus transmission under climate change scenarios. One health (Amsterdam, Netherlands) 2023, 16, 100509. DOI: 10.1016/j.onehlt.2023.100509.

(2) Lv, R.; Lu, N.; Wang, J.; Li, Y.; Qi, Y. Recombinase Polymerase Amplification for Rapid Detection of Zoonotic Pathogens: An Overview. Zoonoses 2022, 2 (1), 163. DOI: 10.15212/ZOONOSES-2022-0002.

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