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N. **Title:**

P41 EVALUATION OF AN ELIME ASSAY TO DETECT HEPATITIS A VIRUS IN DRINKING WATER AND VEGETABLE RINSING WATER

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Abstract:

Background: Waterborne viral infections are recognized as a significant cause of illness worldwide. The monitoring and control of quality and safety of different types of waters, including waters intended for drinking or for use in food preparation, irrigation or recreational activities relies on testing for bacterial indicator microorganisms. However, indicator bacteria have been found unreliable in predicting the presence of enteric viruses, therefore their direct detection is usually required to assess virological safety of waters. Although methods for the detection and quantification of viruses in water are available, they often require specialized laboratory equipment, highly trained personnel and costly reagents. Therefore, the development of simple, rapid, and reliable protocols for detecting viruses in water matrix is required. Aim of this study was the evaluation of an electrochemical sandwich Enzyme Linked Immuno-Magnetic assays (ELIME) for a feasible detection of Hepatitis A virus (HAV) in different water types.

Material and methods: The system is based on the use of goat anti-mouse IgG magnetic beads associated to mouse anti-HAV antibodies as a solid support for the immunochemical chain, and screen-printed electrodes as a sensing platform. Recording of the electrochemical signal involves the use of a portable instrument, able to perform measurements directly in the field (Fig. 1). Assessment of ELIME was performed using two kind of spiked samples: drinking water and vegetable rinsing water (water collected after its use for washing of leafy vegetables in food processing). Two hundred mL of each water type were spiked with HAV (strain HM175) at concentration ranging between 0 and ~10.000 genome copies (g.c.)/mL. Samples were filtered using Zeta Plus 1MDS filters and viruses were eluted with 40 mL of TGBE 0.1% pH 9.5 buffer. After adjusting pH to 7.0 ± 0.2, suspension was divided into two aliquots, for ELIME assay detection (Fig. 2) and for real-time RT-qPCR analysis according to ISO 15216-1.

Results: The ELIME assay detected HAV at the lowest spiking level in both kind of water samples (Table 1 and 2), providing an increased sensitivity compared to the ISO 15216-1 procedure and a detection limit of 0.5 viruses/mL. Linearity of results was modest, particularly on vegetable rinsing water, where organic and disinfectants residues reduced efficiency of both ELIME and PCR analysis. However, underestimation of viral concentration in the spiked samples did not negatively affect the overall ELIME sensitivity.

Conclusions: The described ELIME assay achieved successful detection of HAV in drinking and in vegetable rinsing water. This cost effective and rapid system could be used for virus screening in the field or in food production environments.

Fig. 1: Components of the electrochemical sandwich ELIME assay for HAV detection

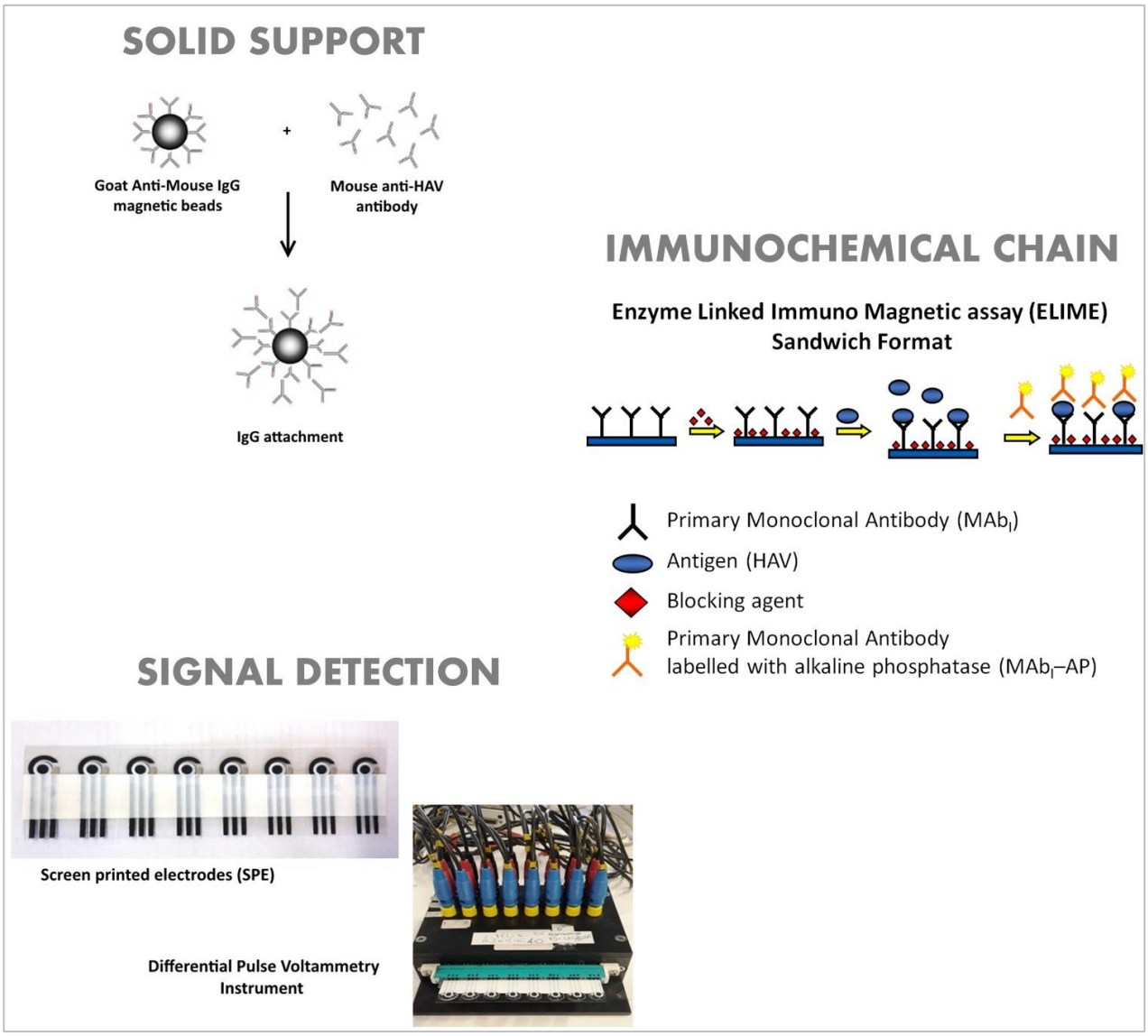

Fig. 2: Protocol used in the ELIME detection

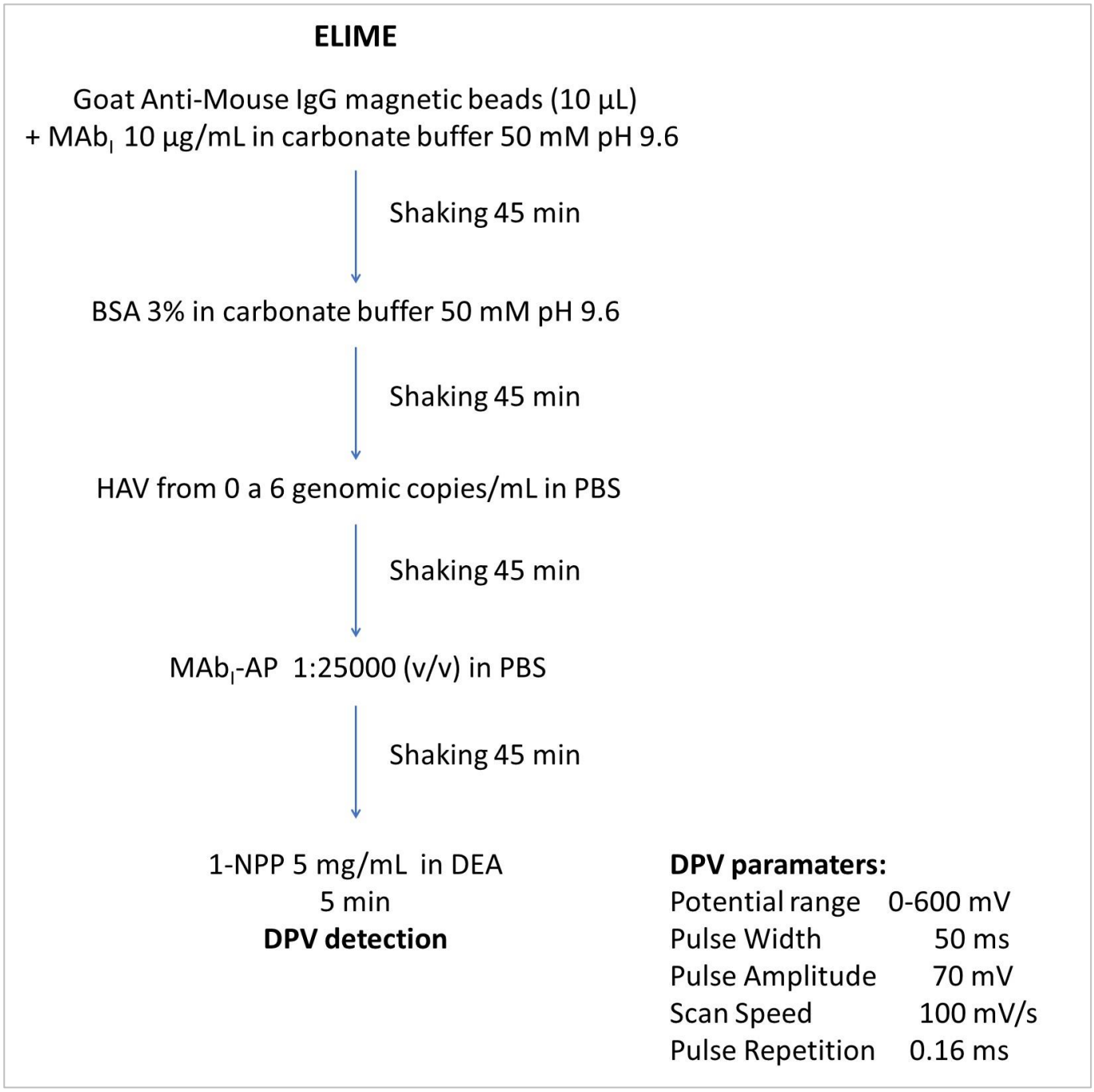

Table 1: Assessment of the ELIME assay on drinking water

Spiking level	HAV expected value (g.c./mL)	ELIME assay (viruses/mL)	ISO 15216-1 real-time RT-qPCR (g.c./mL)
L0	-	-	-
L2	0.82	0.92	-
L2/3	4.11	1.15	0.56 *
L3	8.22	1.45	0.56 *
L3/4	41.08	2.59	2.70
L4	82.16	3.24	4.22
L4/5	410.80	4.42	35.44
L5	821.60	103.54	118.86
L5/6	4107.95	531.06	952.54
L6	8215.96	1088.84	2648.12

* Value <LOQ

Table 2: Assessment of the ELIME assay on vegetable rinsing water

Spiking level	HAV expected value (g.c./mL)	ELIME assay (viruses/mL)	ISO 15216-1 real-time RT-qPCR (g.c./mL)
L0	-	-	-
L2	0.91	1.50	-
L2/3	4.56	1.66	0.19 *
L3	9.12	1.69	0.40 *
L3/4	45.59	1.98	0.53 *
L4	91.18	2.18	0.96 *
L4/5	455.89	3.60	4.04
L5	911.79	30.83	6.46
L5/6	4558.94	135.71	86.85
L6	9117.87	229.09	93.02

* Value <LOQ