EVALUATION OF AN ELIME ASSAY TO REVEAL THE PRESENCE OF HEPATITE A IN DRINKING WATER

C. D'Agostino [1], L. Micheli[1], A. Fasoli[1], E. Suffredini[2], D. Moscone[1]

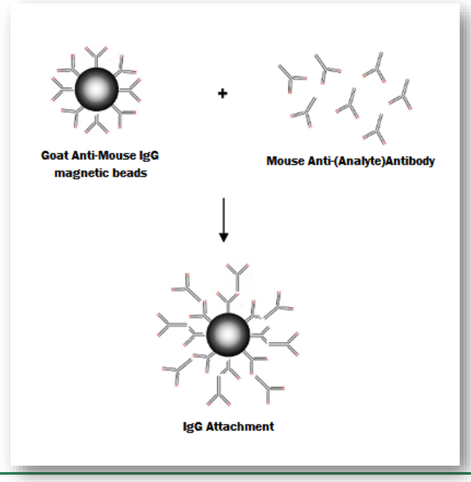
[1]*Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Via della Ricerca Scientifica 1, 00133 Rome, Italy*
[2] *Department of Food Safety,Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy*

Introduction

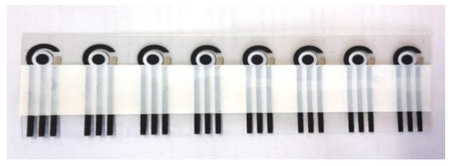
Water-borne viral diseases pose high risks for public health worldwide. Urban wastewaters contain large number of pathogenic viruses, and full removal of virus particles cannot be guaranteed by conventional wastewater treatments. Presently, water quality indicators rely on bacterial fecal indicators, which do not provide adequate information about the presence of pathogenic viruses. Current legislation for microbial contamination in food products and for hygiene in primary production (EC 2073/2005, EC 853/2004, EC 852/2004) does not include any specific provision on enteric viruses in waters used in food production environments or for irrigation purposes. The currently available tests for virus detection, based on molecular biology, are expensive and labor intensive, thus limited to laboratories with suitable equipment and well-trained personnel. In this work, a cost effective and rapid system for Hepatitis A virus (HAV) monitoring in different freshwater bodies is designed. An electrochemical sandwich **Enzyme Linked Immuno Magnetic** assay (ELIME) is proposed [1]. The system is based on the use of Goat Anti-Mouse IgG magnetic beads as solid support for the immunochemical chain, and screen-printed electrodes as a sensing platform. Using these ELIME assays, a quantitative determination of HAV can be achieved with a detection limit of 0.5 genome copies /mL. The proposed system was successfully applied to detect HAV in drinking water. Results obtained on spiked samples were compared to those obtained by the standardized qRT-PCR analysis (ISO 15216-1) commonly applied to assess HAV presence in water samples.

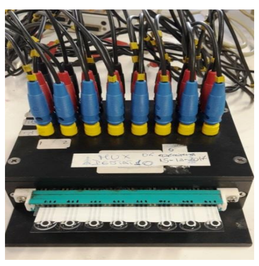
METHODS AND MATERIALS

Goat Anti-Mouse IgG magnetic beads



Screen Printed Electrode (SPE)

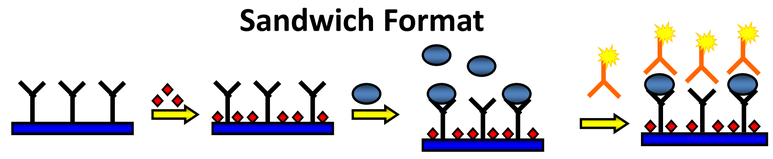




DPV paramaters:

-Potential range	0-600 mV
-Pulse Width	50 ms
-Pulse Amplitude	70 mV
-Scan Speed	100 mV/s
-Pulse Repetition	0.16 ms

Enzyme Linked Immuno Magnetic assay (ELIME) Sandwich Format



Primary monoclonal antibody (MAb_I)

Antigen (HAV)

Blocking agent

Primary monoclonal antibibody labelled with alkaline phosphatase (MAb_I –AP)

Extraction method

100 mL drinking water

Filtration on Zeta Plus™ 1MDS

Eluition with 40 mL TGBE 0.1% pH 9.5

Shaking 45 min

Adjust at pH 7

ELIME | Concentration by Amicon

RNA extraction (100 µL)

Real Time RT-PCR (10 µL)

ELIME

Goat Anti-Mouse IgG magnetic beads (10 µL)
+ MAb_I 10 µg/mL in carbonate buffer 50 mM pH 9.6

Shaking 45 min

BSA 3% in carbonate buffer 50 mM pH 9.6

Shaking 45 min

HAV from 0 a 6 genomic copy num./mL in PBS

Shaking 45 min

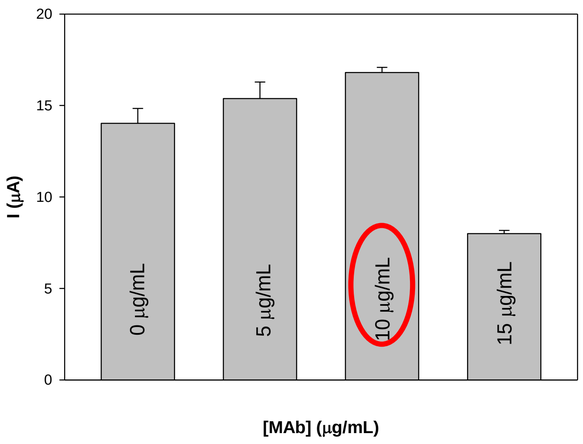
MAb_I-AP 1:25000 (v/v) in PBS

Shaking 45 min

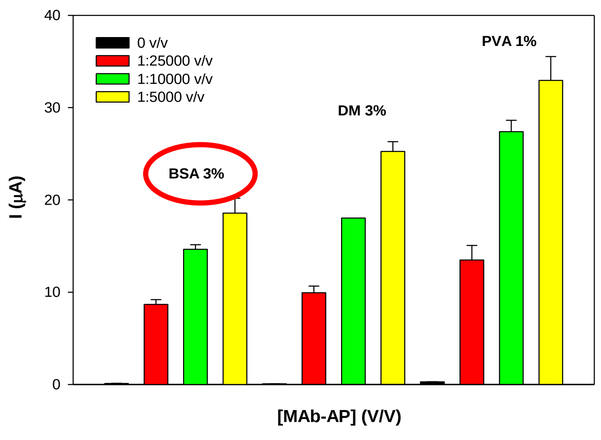
1-NPP 5 mg/mL in DEA
5 min
DPV detection

RESULTS

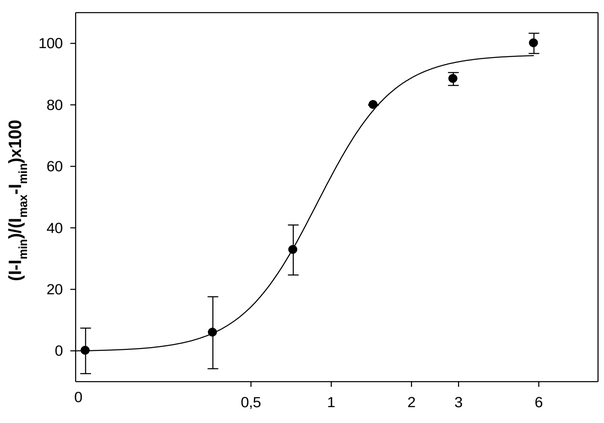
Binding: study of MAb_I concentration



Study of blocking agent



Calibration curve



LOD	0.5 genomic copy/mL
RSD %	7 %
Range	from 0.4 to 6 genomic copy/mL

Vegetables washing water samples: comparison between ELIME assay and qRT-PCR

	VIRUS	ELIME	PCR	
Level	**Expected value PCR (gen.cop./mL)**	**Eluate in TGBE 0.1% ELIME (gen.cop./mL)**	**Eluate in TGBE 0.1% PCR (gen.cop./mL)**	**Concentrated by Amicon PCR (gen.cop./mL)**
L0	nd	0	nd	nd
L2	0.91	1.50	nd	nd
L2/3	4.56	1.66	nd	0.19
L3	9.12	1.69	nd	0.40
L3/4	45.59	1.98	13.2	0.53
L4	91.18	2.18	16.4	0.96
L4/5	455.89	3.60	32.4	4.04
L5	911.79	30.83	62.8	6.46
L5/6	4558.94	135.71	518.8	86.85
L6	9117.87	229.09	536.4	93.02

CONCLUSIONS

-A rapid, sensitive and low-cost analysis method, the sandwich ELIME, for determination of HAV was developed with detection limit of 0.5 genomic copy number/mL;

-An evaluation of HAV contamination levels in samples of different freshwater was carried out;

-The results obtained with the application of ELIME and RT-PCR for analyzing the same fortified water samples underlined that the proposed technique is able to detect lower concentration of RT-PCR with good sensitivity and reproducibility.

[1] L. Micheli, A. Fasoli, A. Attar, D. T. Donia, M. Divizia, A. Amine, G. Palleschi, P. A. Salazar Carballo, D. Moscone (2017) Procedia Technology, 27, 85-86.