

Supplementary Material

# Dual-Modal Assay Kit for the Qualitative and Quantitative Determination of the Total Water Hardness Using a Permanent Marker Fabricated Microfluidic Paper-Based Analytical Device

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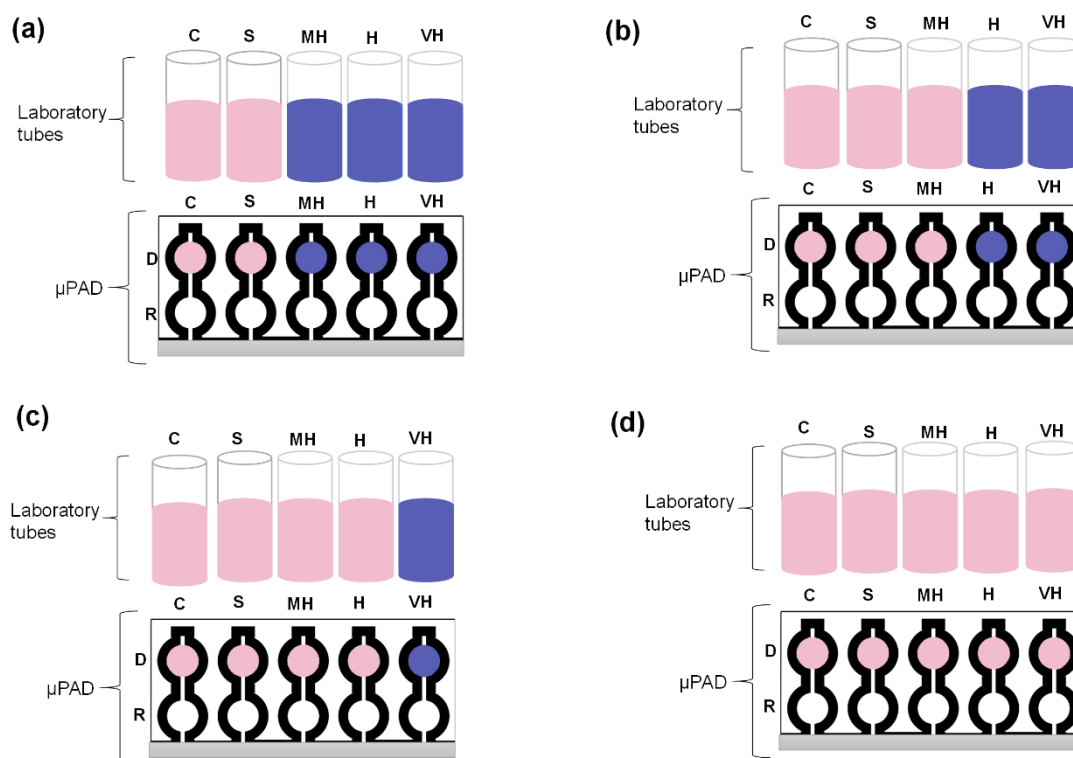
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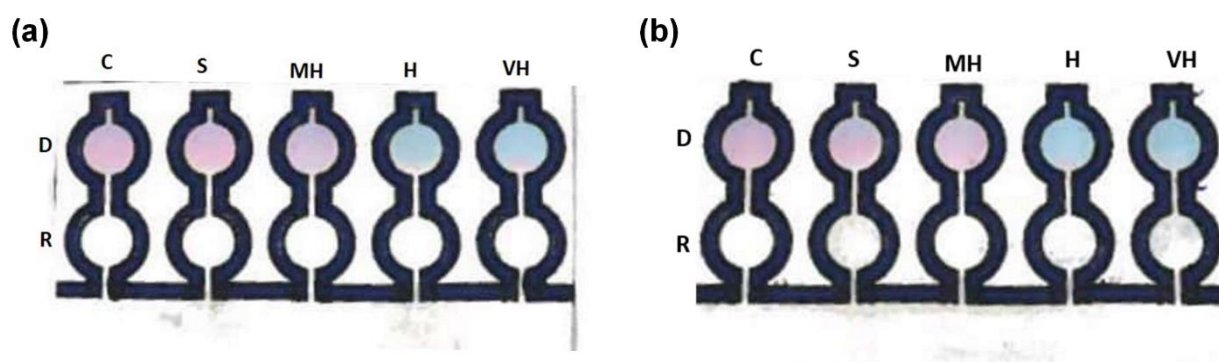
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**Table S1.** Calculations of the stoichiometric expectations for the test results of the spiked water samples with Mg<sup>2+</sup> and Ca<sup>2+</sup> using the paper-based device. (a) Soft water with the total hardness less than 60 mM; (b) moderately hard water with the total hardness between 0.61-1.20 mM; (c) hard water with the total hardness between 1.21-1.8 mM; (d) very hard water with the total hardness more than 1.81 mM.

Channel	Hardness of the water sample (mM) "Volume = 11 $\mu$ L"	Concentration of the applied EDTA (mM) in the reaction (R) zone "volume = 5.5 $\mu$ L"	Hardness of water moving to the detection (D) zones (mM)	Color output at the detection (D) zones
<i>a) Soft water</i>				
Channel 1 (C)	0.40	0.00	0.40	Pink
Channel 2 (S)	0.40	0.40	0.20	Pink
Channel 3 (MH)	0.40	1.22	0.00	Blue
Channel 4 (H)	0.40	2.42	0.00	Blue
Channel 5 (VH)	0.40	3.62	0.00	Blue
<i>b) Moderately hard water</i>				
Channel 1 (C)	0.81	0.00	0.81	Pink
Channel 2 (S)	0.81	0.40	0.61	Pink
Channel 3 (MH)	0.81	1.22	0.20	Pink
Channel 4 (H)	0.81	2.42	0.00	Blue
Channel 5 (VH)	0.81	3.62	0.00	Blue
<i>c) Hard water</i>				
Channel 1 (C)	1.40	0.00	1.40	Pink
Channel 2 (S)	1.40	0.40	1.20	Pink
Channel 3 (MH)	1.40	1.22	0.79	Pink
Channel 4 (H)	1.40	2.42	0.19	Pink
Channel 5 (VH)	1.40	3.62	0.00	Blue
<i>d) Very hard water</i>				
Channel 1 (C)	2.00	0.00	2.00	Pink
Channel 2 (S)	2.00	0.40	1.80	Pink
Channel 3 (MH)	2.00	1.22	1.39	Pink
Channel 4 (H)	2.00	2.42	0.79	Pink
Channel 5 (VH)	2.00	3.62	0.19	Pink



**Figure S1.** Schematic of the complexometric titration in the solution phase in laboratory test tubes and the expectations on the paper-based device according to the stoichiometric calculations which were presented in Table S1: (a) soft water, (b) moderately hard water, (c) hard water, and (d) very hard water (D: detection zone; R: reaction zone; C: control; S: soft; MH: moderately hard; H: hard; VH: very hard).



**Figure S2.** Qualitative detection of total hardness of water containing only a single ion of calcium and magnesium ion. (a) 0.81 mM calcium ion, and (b) 0.81 mM magnesium ion.

#### Maximum allowable concentration (MAC) of ions in water

The maximum allowable concentration of some common ions in water includes; manganese ion (2.19  $\mu\text{M}$ ), iron (II) ion (5.38  $\mu\text{M}$ ), ammonium ion (5.56  $\mu\text{M}$ ), chloride ion (56.34  $\mu\text{M}$ ), fluoride ion (78.95  $\mu\text{M}$ ) and copper ion (31.25  $\mu\text{M}$ ) [1].

## References

1. Health Canada. Guidelines for Canadian Drinking Water Quality—Summary Table. Available online: <https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality/guidelines-canadian-drinking-water-quality-summary-table.html> (accessed on 7 October 2020).