Supplementary Information (SI) for Analyst.
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Supplementary material for

Integration of paper-based analytical devices with digital microfluidics for colorimetric detection of creatinine

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Materials and methods

Preparation of artificial urine:

The artificial urine solution was prepared with ultrapure water by mixing the substances at the concentrations described in Table S1

Table S1 Composition of artificial urine solution.

Components	Concentration (mmol L^{-1})
Ammonium chloride	25.0
Calcium chloride	2.5
Citric acid	2.0
Dibasic sodium phosphate	7.0
Lactic acid	1.1
Magnesium sulfate	2.0
Potassium phosphate monobasic	7.0
Sodium bicarbonate	2.5
Sodium chloride	9.0
Sodium sulfate	10.0
Urea	170

Wax printing of paper-based analytical devices:

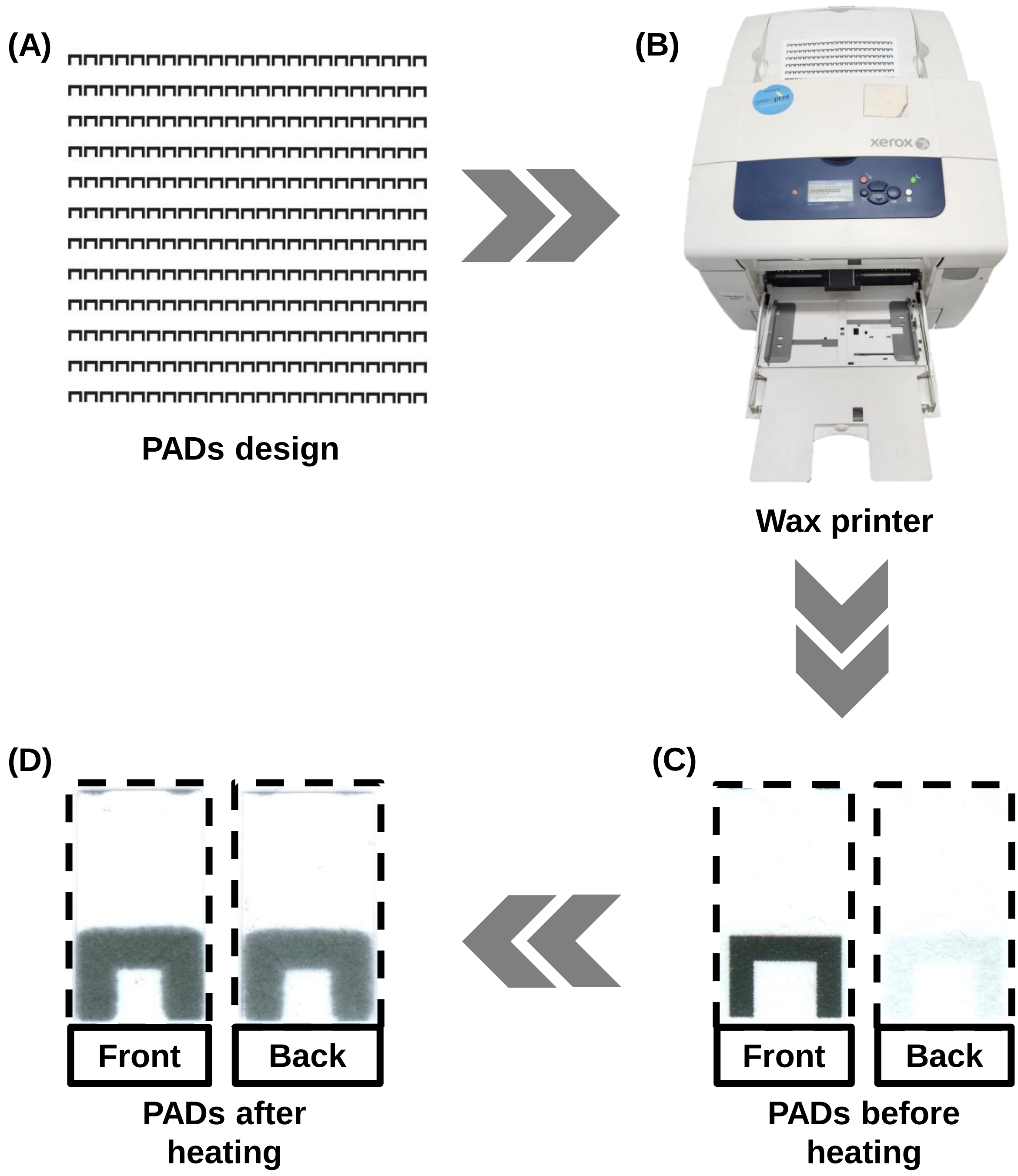


Fig. S1 Schematic representation of the manufacturing of PADs. In (A) the design of PADs for a sheet of paper, (B) wax printer, (C) front and back of a PAD before heating, (D) front and back of a PAD after heating.

Integration of DMF and PADs:

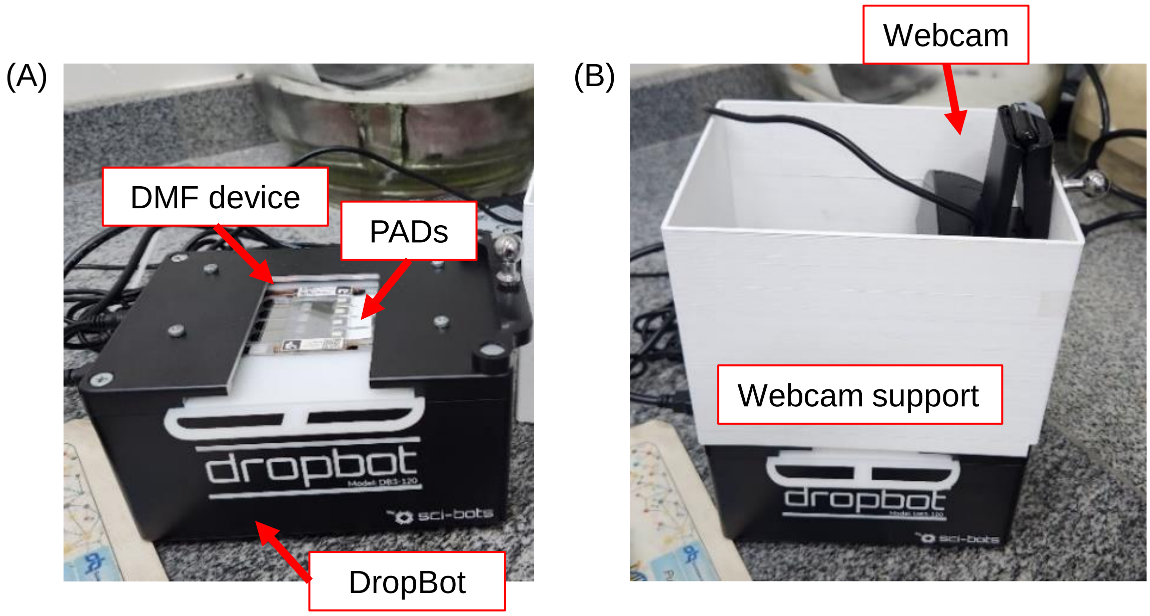


Fig. S2 (A) DropBot equipment with the DMF chip integrated with PADs. (B) DropBot equipment with a removable 3D printer frame to support the webcam.

Video S1 Video of the reaction automated protocol.

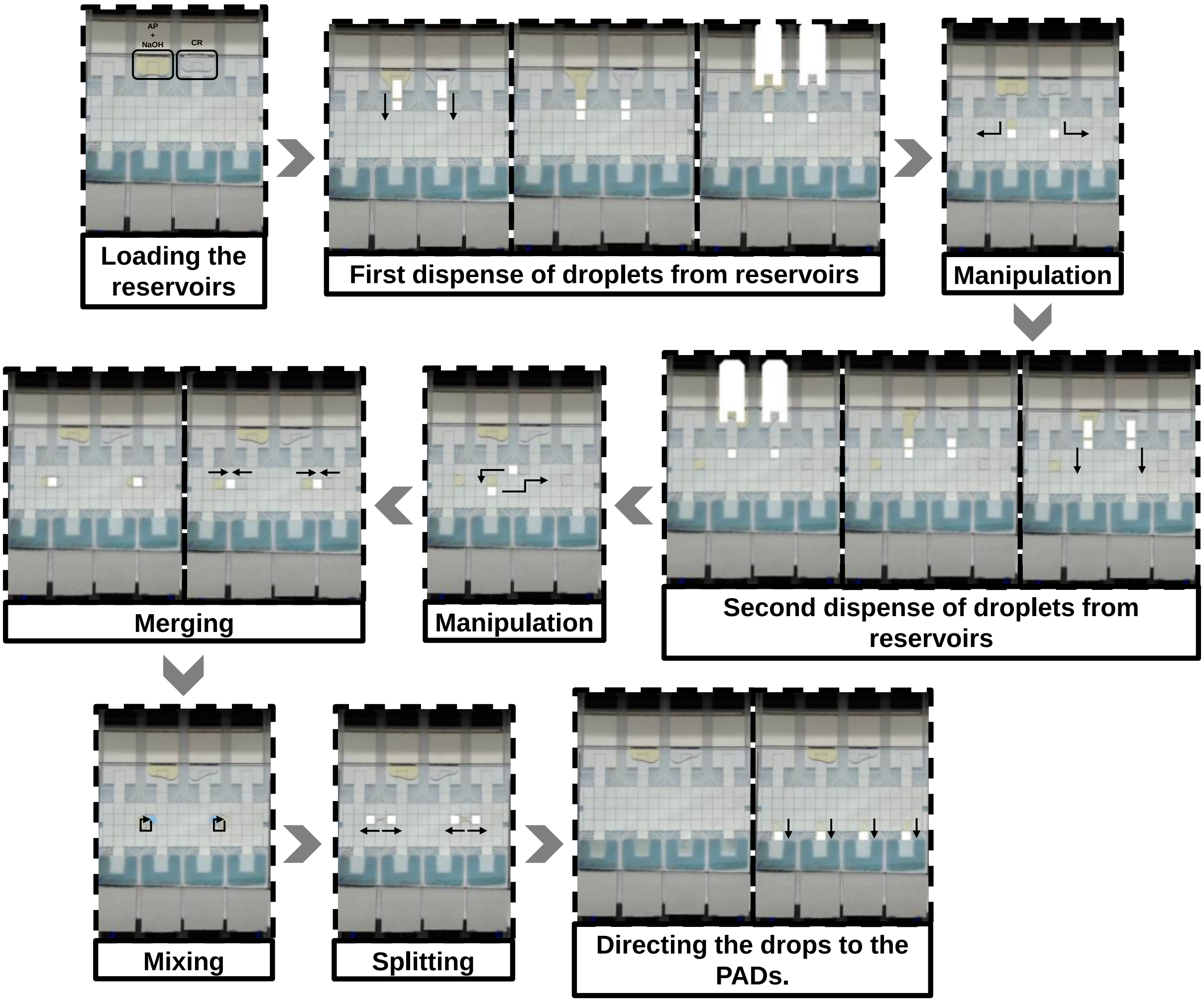


Fig. S3 Simplified scheme of the steps performed in the DMF automated Jaffé reaction protocol.

Video S2 Video of dilutions of synthetic urine samples.

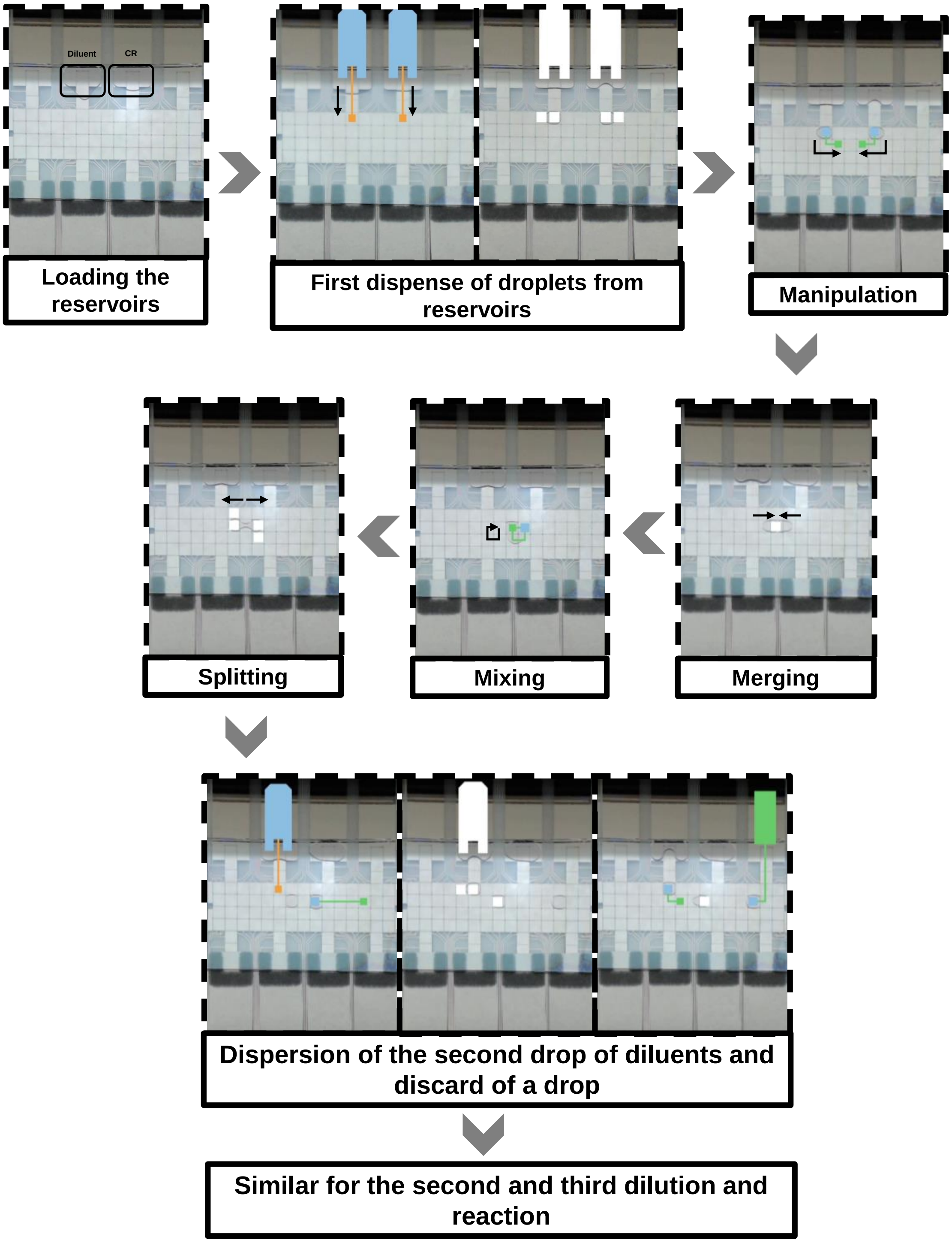


Fig. S4 Simplified scheme of the dilution protocol for artificial urine spiked with known concentrations of creatinine. After the third dilution no droplets are discarded.

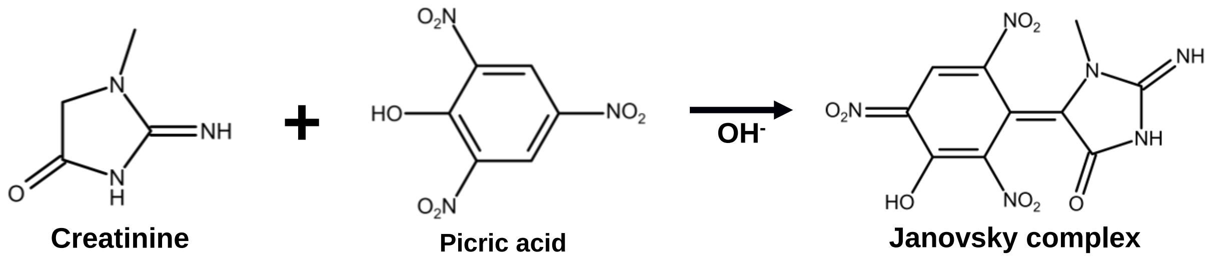


Fig. S5 The scheme of the Jaffé reaction, which consists of the reaction between creatinine and picric acid (yellow) under alkaline conditions to form the Janovsky complex (orange)**.**

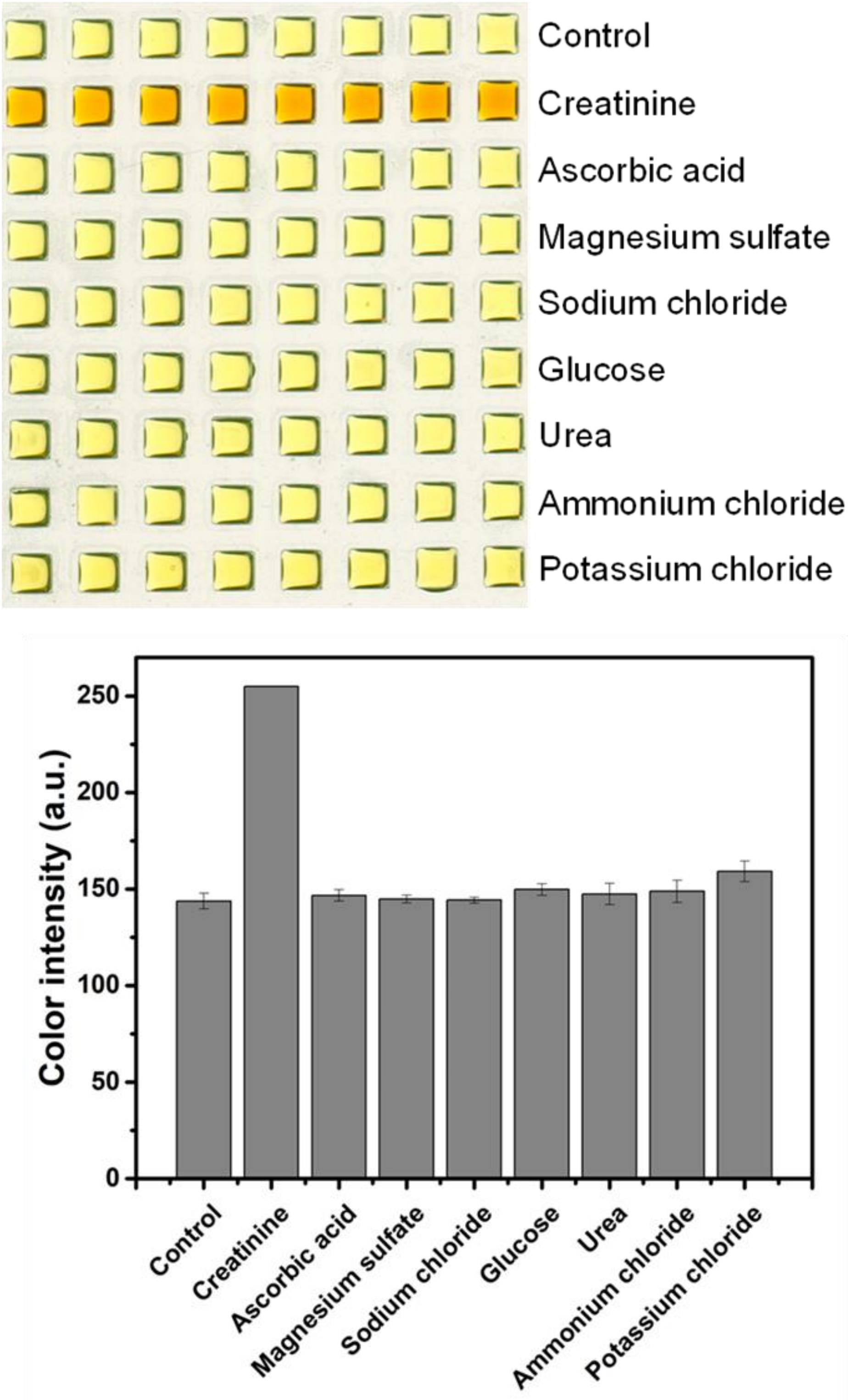


Fig. S6 Evaluation of potential interfering compounds on CR detection. Top: Image of tests carried out on the polyester plate. Bottom: graph of the average color intensities obtained for several potential compounds present urine interrogated with the Jaffé reaction. Error bars represent ± standard deviation, n =8.

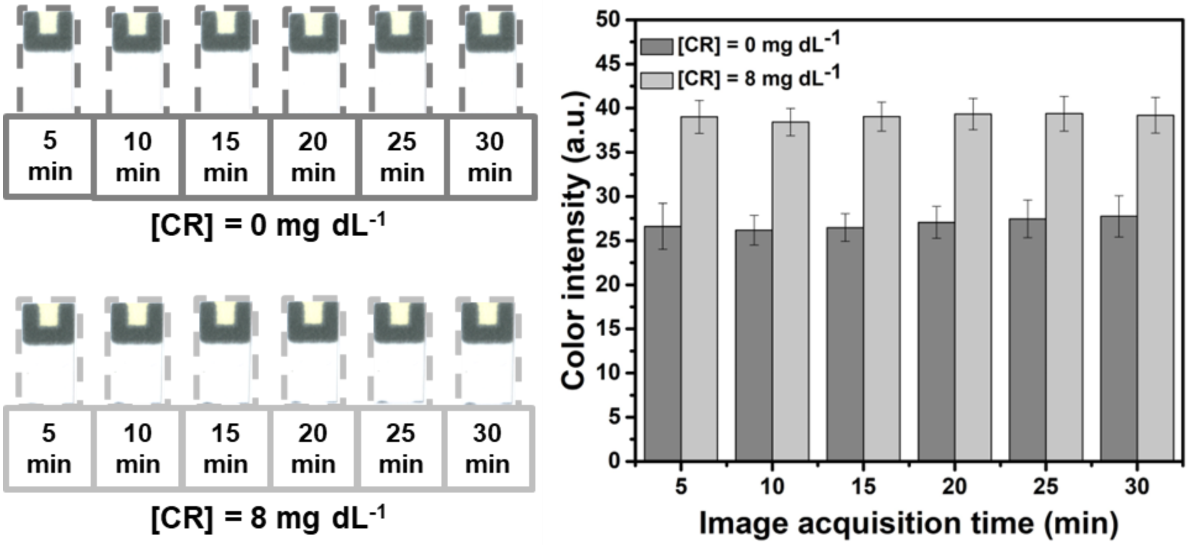
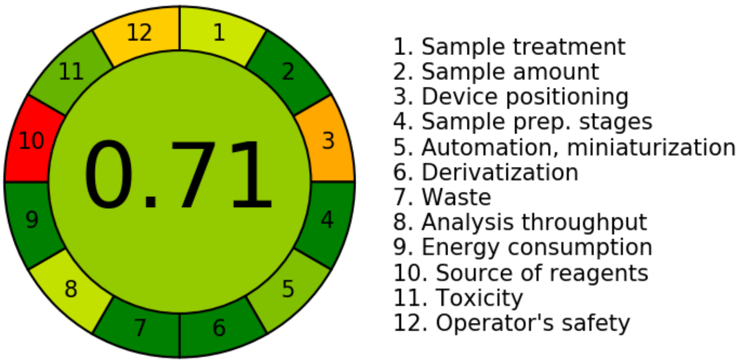


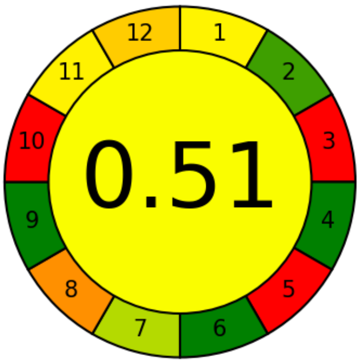
Fig. S7 PADs and time optimization graph for image acquisition by color intensity. Error bars represent ± standard deviation, n =4.

DMF-PAD



Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.6	2
2. Minimal sample size and minimal number of samples are goals.	1.0	2
3. If possible, measurements should be performed in situ.	0.33	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	0.75	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	1.0	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.62	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	0.0	2
11. Toxic reagents should be eliminated or replaced.	0.8	2
12. Operator's safety should be increased.	0.4	2

Reference analysis via UV-Vis



1. Sample treatment
2. Sample amount
3. Device positioning
4. Sample prep. stages
5. Automation, miniaturization
6. Derivatization
7. Waste
8. Analysis throughput
9. Energy consumption
10. Source of reagents
11. Toxicity
12. Operator's safety

Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.48	2
2. Minimal sample size and minimal number of samples are goals.	0.88	2
3. If possible, measurements should be performed in situ.	0.0	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	0.0	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.65	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.29	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	0.0	2
11. Toxic reagents should be eliminated or replaced.	0.48	2
12. Operator's safety should be increased.	0.4	2

Fig. S8 Pictogram obtained through an analysis of the AGREE metric, comparing the DMF-PAD method with the reference analysis via spectrophotometry.