



Validation of a new multiplex dipstick for the simultaneous detection of sulfonamides, fluoroquinolones, tylosin and chloramphenicol in honey



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I. Introduction

- The use of antibiotics in apiculture is not permitted within Europe.
- There are no EU Maximum Residue Limits (MRLs) for any antimicrobials in honey; but...
 - A Minimum Required Performance Limit (MRPL) of 0.3 µg kg⁻¹ has been fixed for chloramphenicol (EU Commission, 2003)
 - 'Recommended concentrations' range from 20 µg kg⁻¹ for tylosin to 50 µg kg⁻¹ for sulfonamides (CRL Guidance Paper, 2007)
 - These concentrations have been taken into account in setting the Screening Target Concentrations (STCs). The STC is the concentration at which a screening test categorises the sample as "Screen Positive" (potentially non-compliant) and triggers a confirmatory test.
- Because of concerns about the occurrence of antibiotics in honey, a rapid dipstick method has been developed and validated at the relevant STC concentrations, at least for sulfonamides, fluoroquinolones and tylosin.

II. Materials and Methods

Materials and Reagents

The Heatsensor®, Readsensor®



and the multiplex dipstick kits were provided by Unisensor s.a. (Liège, Belgium).



Method Principle

	A) SAMPLE "hydrolysed" (SULFA)	B) SAMPLE "protected" (TYL-A)
HONEY SAMPLE	2.5 g (50 ml tube)	2.5 g (50ml tube)
DISSOLUTION	1.2 ml "ACID buffer" (mix) → 5 min at 95°C 1.2 ml "NEUTRALIZING buffer"	2.4 ml H ₂ O → DISSOLUTION at 40°C, 20 min
EXTRACTION	10 ml ETHYL ACETATE Shake 10 min Centrifuge 5 min (4500rpm)	
SUPERNATANT	Transfer 8 ml supernatant into 2 new 15 ml tube	
EVAPORATION	50 +/- 5°C (N ₂) for 50 min until dryness	
RECONSTITUTION	Extract "A" reconstituted in 250µl "DIPSTICK buffer"	Extract "B" reconstituted in 250µl "DIPSTICK buffer"
DIPSTICK ANALYSIS	Mix extracts "A" and "B" (1:1) Add 200 µl of the MIX to the microwell → 5 min at 40°C Add the DIPSTICK to the microwell → 10 min at 40°C → remove filter → Readsensor®	

Interpretation of Results (figure 1)

- Samples that 'screen positive' (potentially non-compliant) will have the same or a reduced/weaker red colour (compared to the control line) at the test line.
- In the case of compliant (or 'negative') samples the test line will be more intense in colour than the control line.

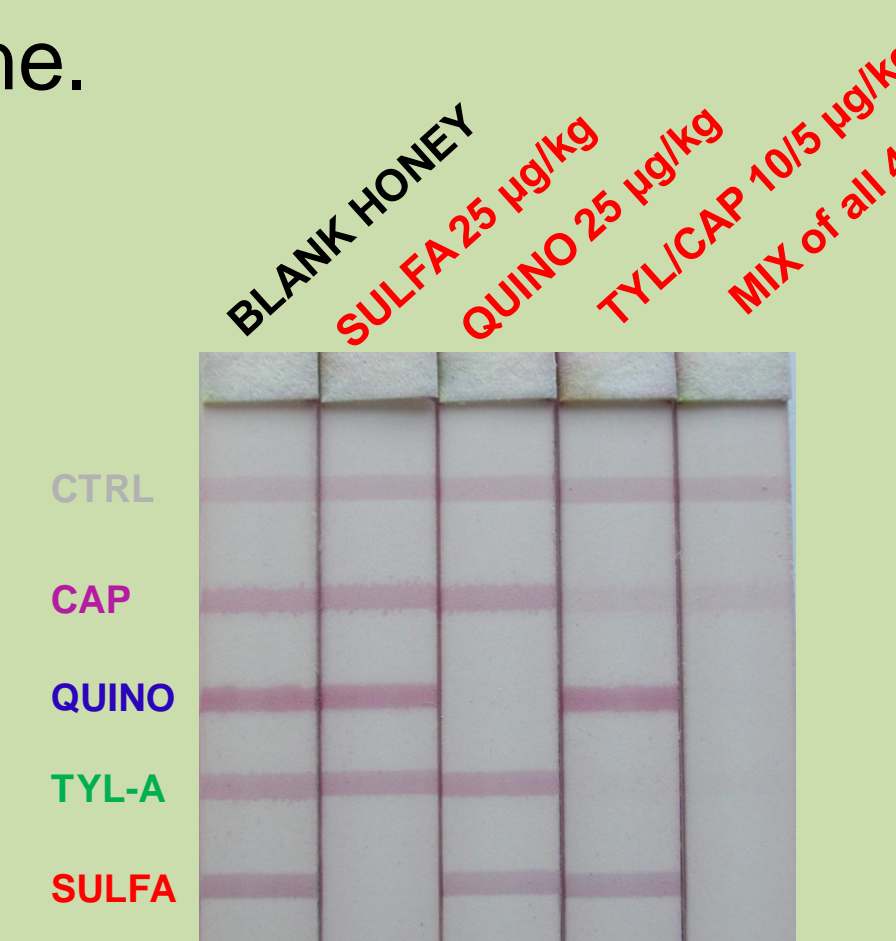


Figure 1 : Example of multiplex dipstick test lines

III. Results

Detection capability and Ruggedness

- 60 'blank' honey samples were spiked at STCs of 50, 50, 20 and 10 µg kg⁻¹ for sulfonamides (sulfathiazole), fluoroquinolones (ciprofloxacin), tylosin-A, and chloramphenicol, respectively.
- All samples were correctly identified as positives by both visual and Readsensor® measurements.
- Further 21 different honey samples (taken from either retail shops or hives) were spiked at ½ STC and STC for all four analytes.
- The CCβ of the method was deemed to be ≤ 25, 25, 10, and 5 µg kg⁻¹ for sulfonamides (sulfathiazole), fluoroquinolones (ciprofloxacin), tylosin, and chloramphenicol, respectively.

Specificity

- No cross reactivity observed with other compounds used in beekeeping husbandry.

Overview of Limits of Detection (LOD)

Sulfonamides	LOD [µg kg ⁻¹]	Fluoroquinolones	LOD [µg kg ⁻¹]
Sulfathiazole	25	Ciprofloxacin	25
Sulfadiazine	25	Danofloxacin	25
Sulfapyridine	25	Enrofloxacin	10
Sulfamerazine	25	Nalidixic acid	25
Sulfamethazine	25	Norfloxacin	10
Sulfamethoxyypyridazine	25	Oxolinic acid	25
Sulfachloropyridazine	25	Flumequine	100
Sulfamonomethoxine	25	Marbofloxacin	100
Sulfadimethoxine	25	Difloxacin	100
Sulfamoxole	25		
Sulfaquinoxaline	25		
		Others	LOD [µg kg ⁻¹]
		Tylosin	10
		Chloramphenicol	5

IV. Conclusion

- This single laboratory validation of the multiplex dipstick method demonstrates the detection capability of sulfonamides, fluoroquinolones, tylosin and chloramphenicol in honey.
- This multiplex dipstick assay (BEE4SENSOR®) is rapid, convenient and is applicable for the screening of antibiotics in honey samples at different quality control points along the supply chain.

V. Outlook

- An inter-laboratory validation has followed and the outcome will be presented elsewhere.
- A simplified format of the protocol has been developed and validated for field-testing.
- This field-test format will be evaluated by a minimum of 20 bee-inspectors, during the honey-flow season 2012 in the UK.

References

- European Commission (2003). Commission Decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin. L71. 17-18.
- CRL Guidance paper (2007). <http://www.rivm.nl/bibliotheek/digitaaldepot/crlguidance2007.pdf>

