
BEE4SENSOR : a new multiplex dipstick for the simultaneous detection of sulfonamides, (fluoro)quinolones, tylosin and chloramphenicol in honey

DEMO for WP2b/WP2a open day EURORESIDUE VII

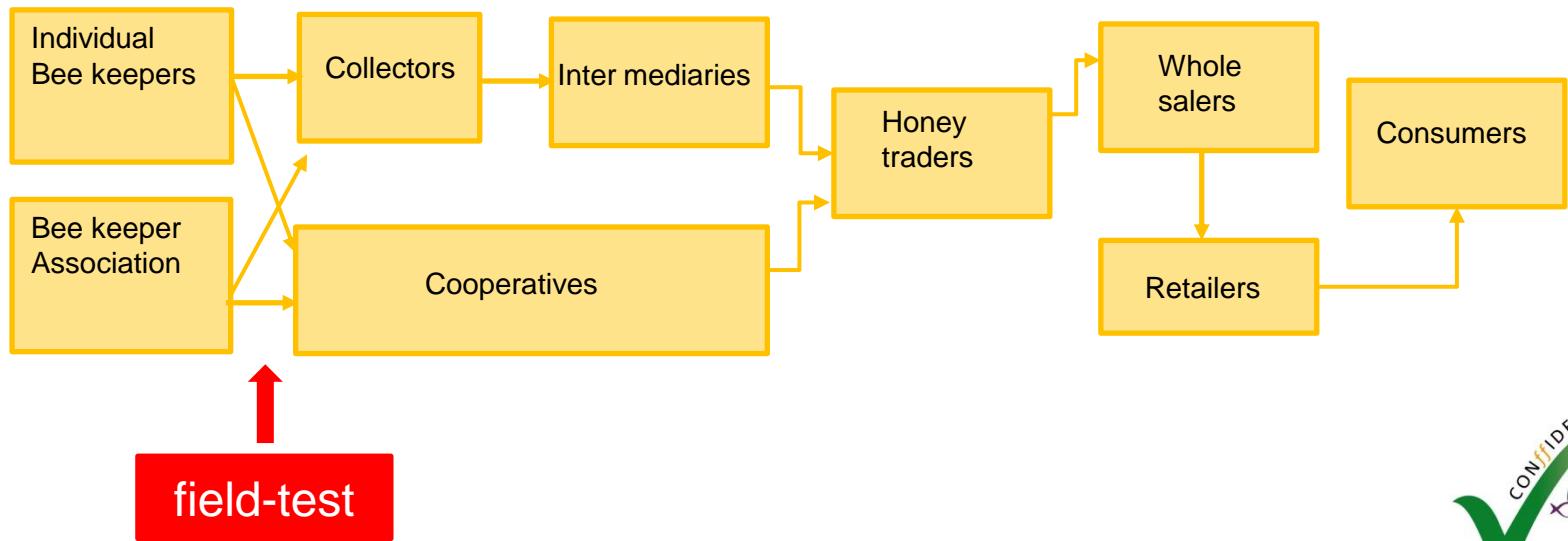
The Netherlands, 16th MAY 2012



Assay format :

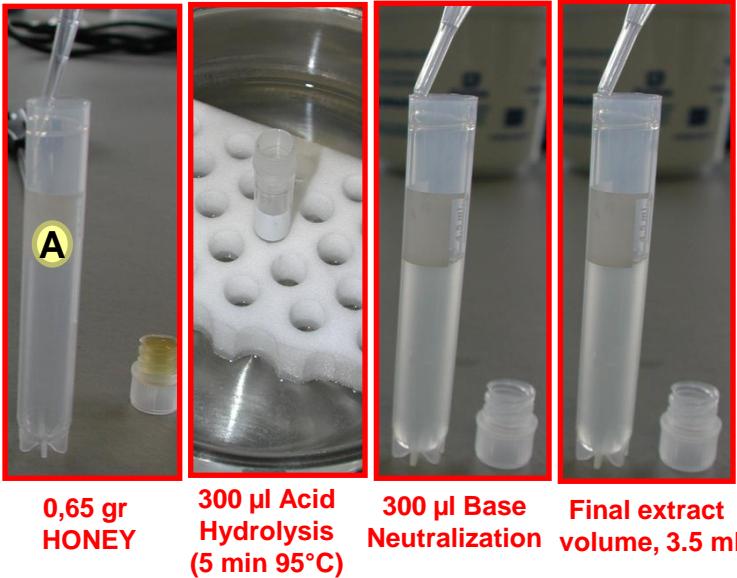
➤ field-test assay

- no lab equipment required ;
- less sensitive than lab-test assay, but sufficient to detect contaminated batches from treated hives ;
- suited for collectors, cooperatives to test individual lots from beekeepers.

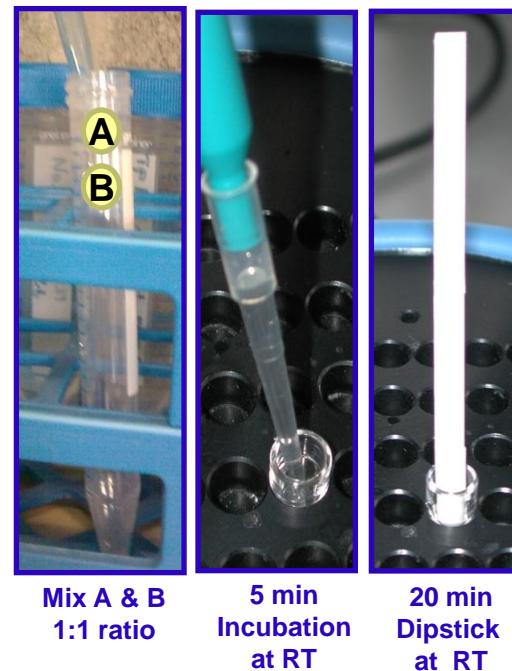


Field-test assay : method schematic

1. HYDROLYSIS / DILUTION

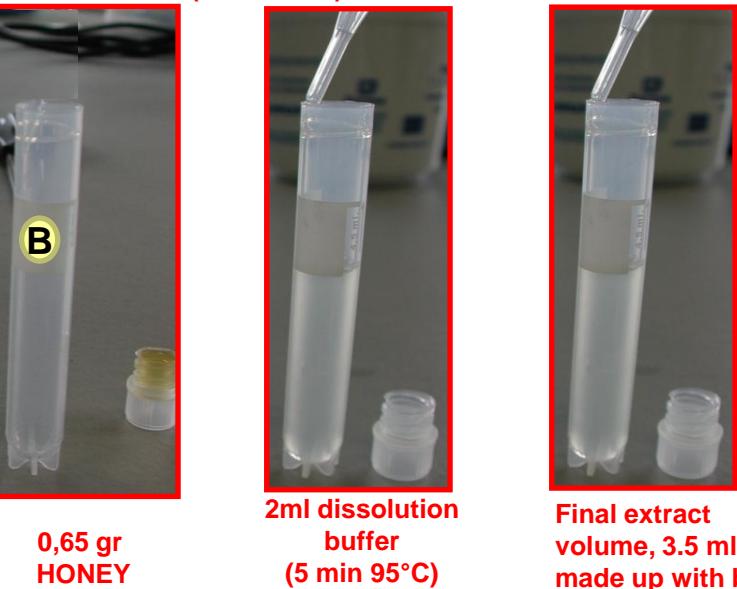


2. DIPSTICK



<30 min
TOTAL

All material provided in the kit





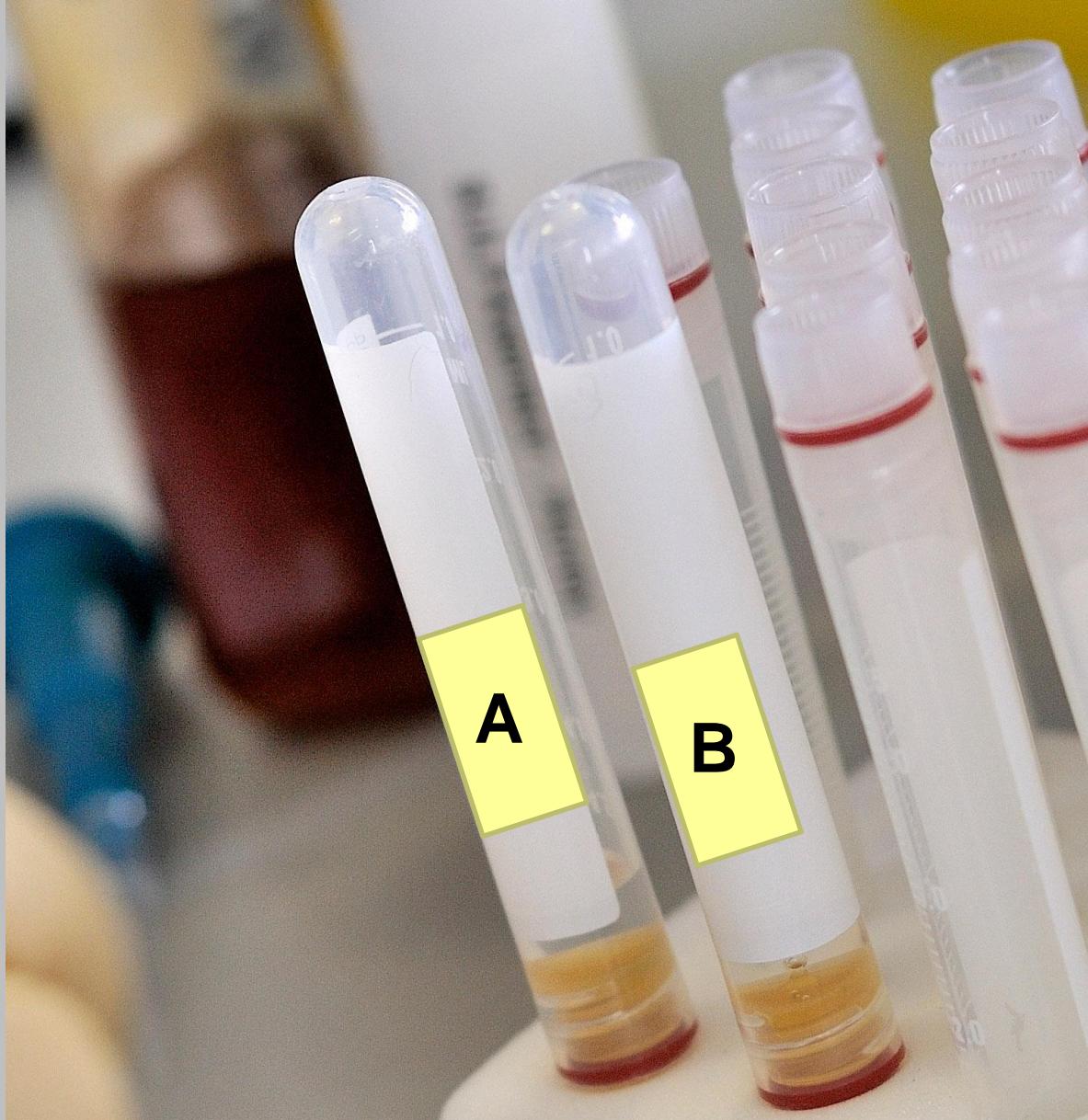
Kit content



~650 mg Honey / sample

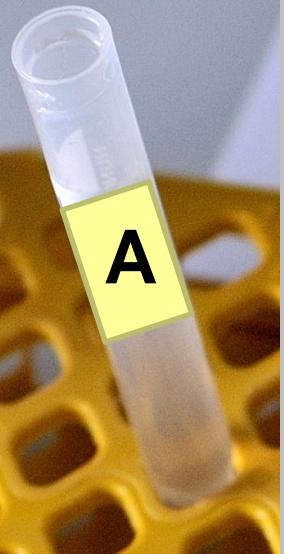




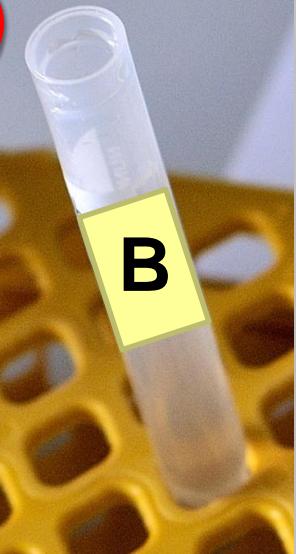


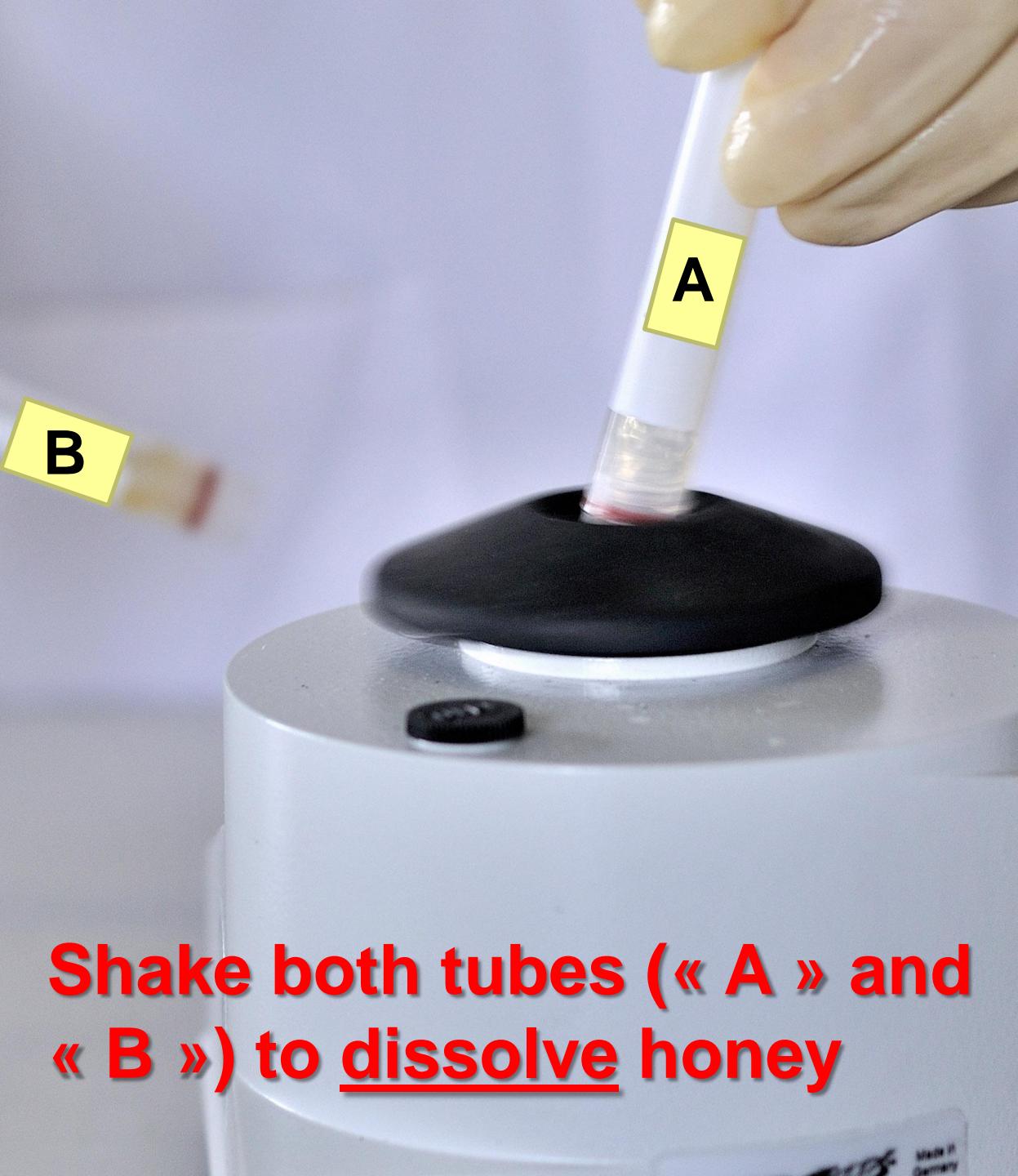
Prepare 2 aliquots / sample

Add 6 drops of
Acid Buffer (300µl)
In tube « A »

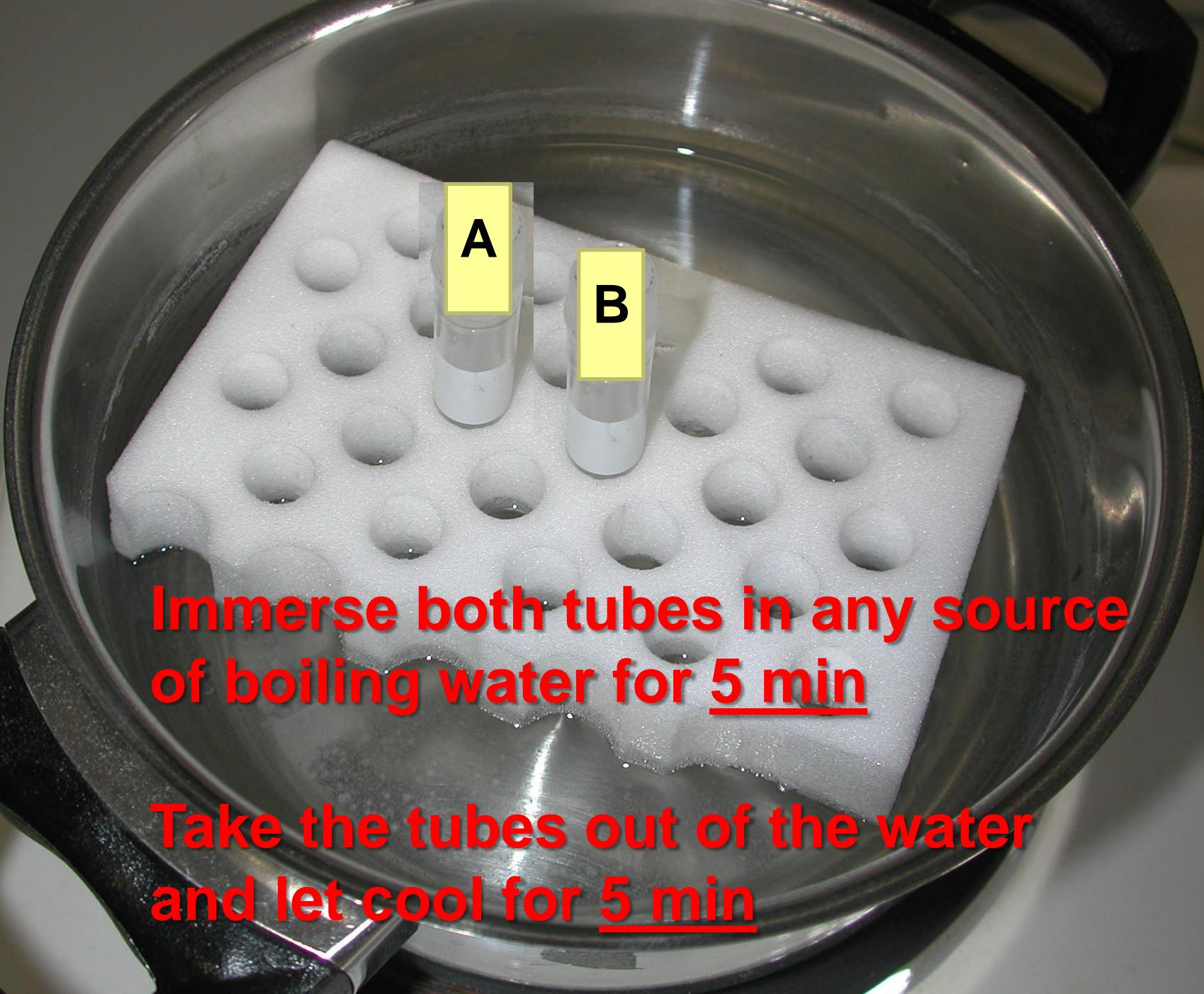


Adjust tube « B »
to ~2 ml with Buffer
(add ~1.5 ml)





Shake both tubes (« A » and « B ») to dissolve honey



**Immerse both tubes in any source
of boiling water for 5 min**

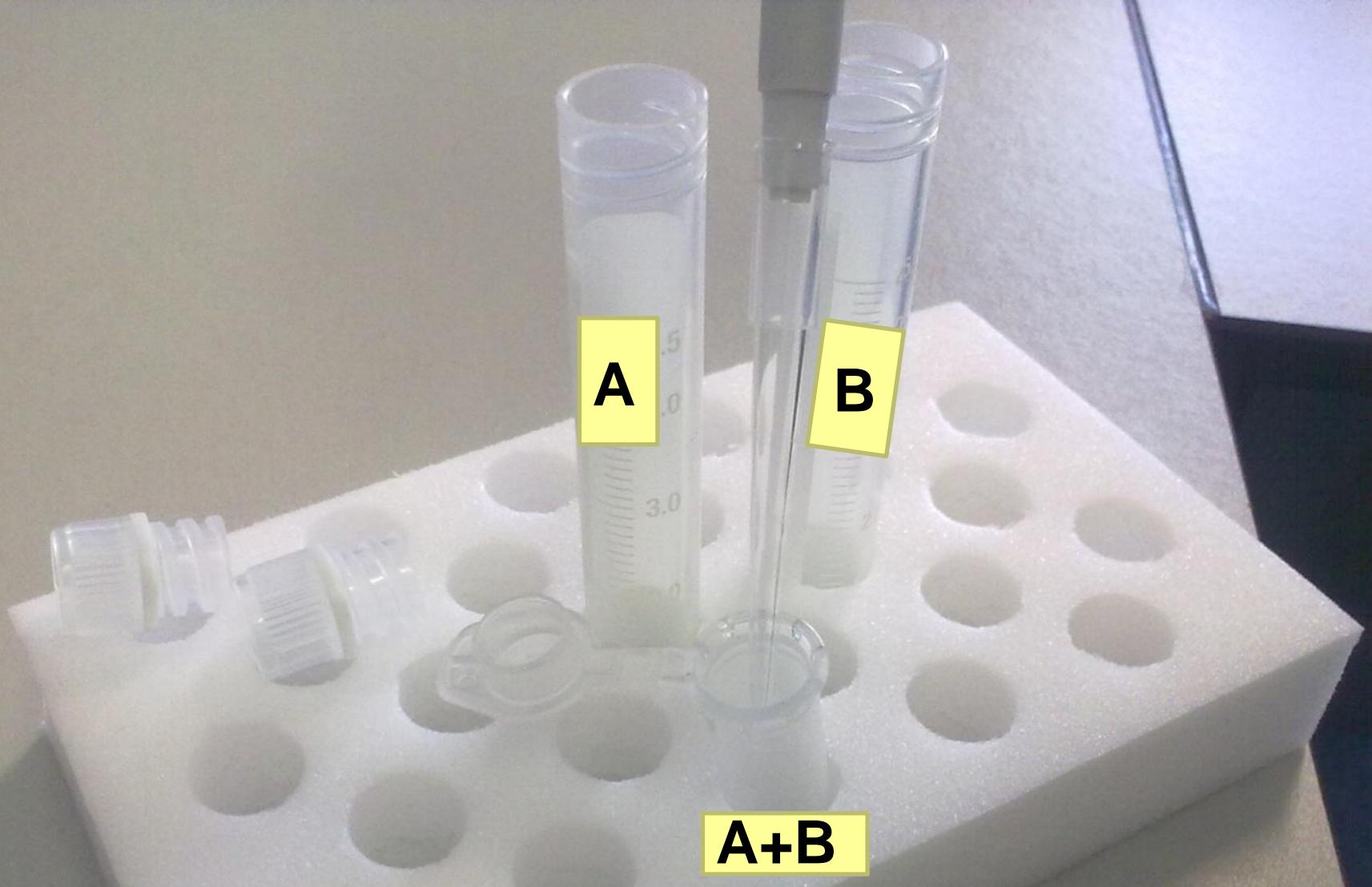
**Take the tubes out of the water
and let cool for 5 min**

**Add 6 drops of
Neutralizing Buffer
(300µl) in tube « A »**

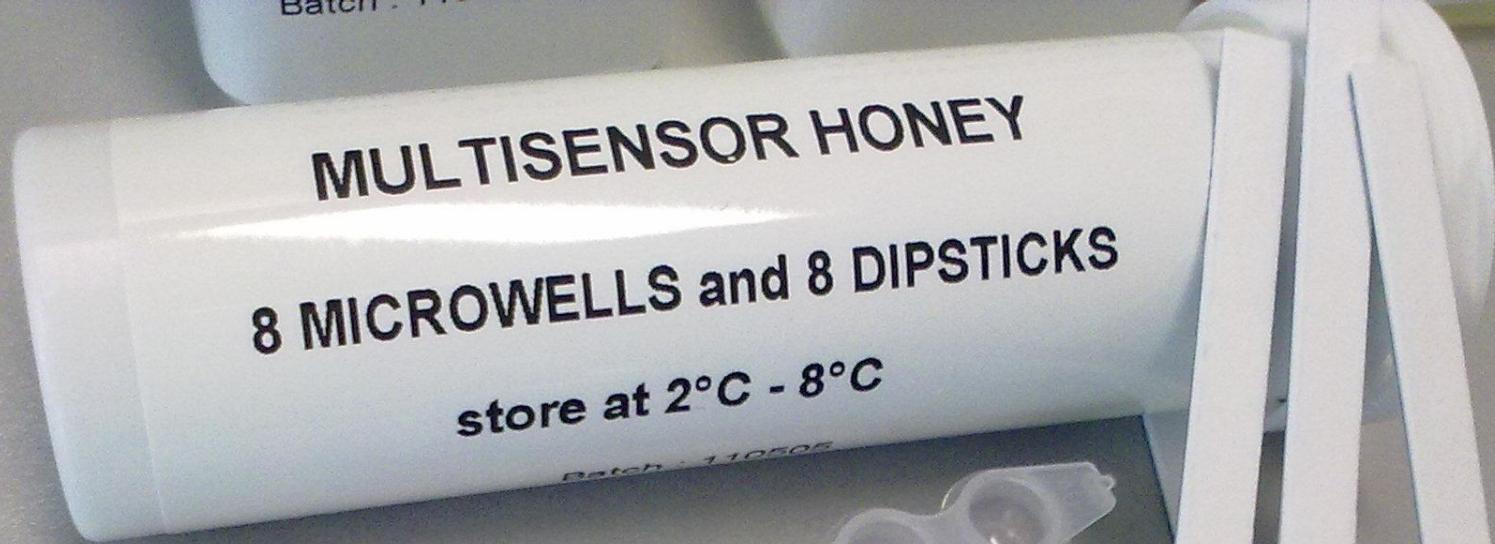




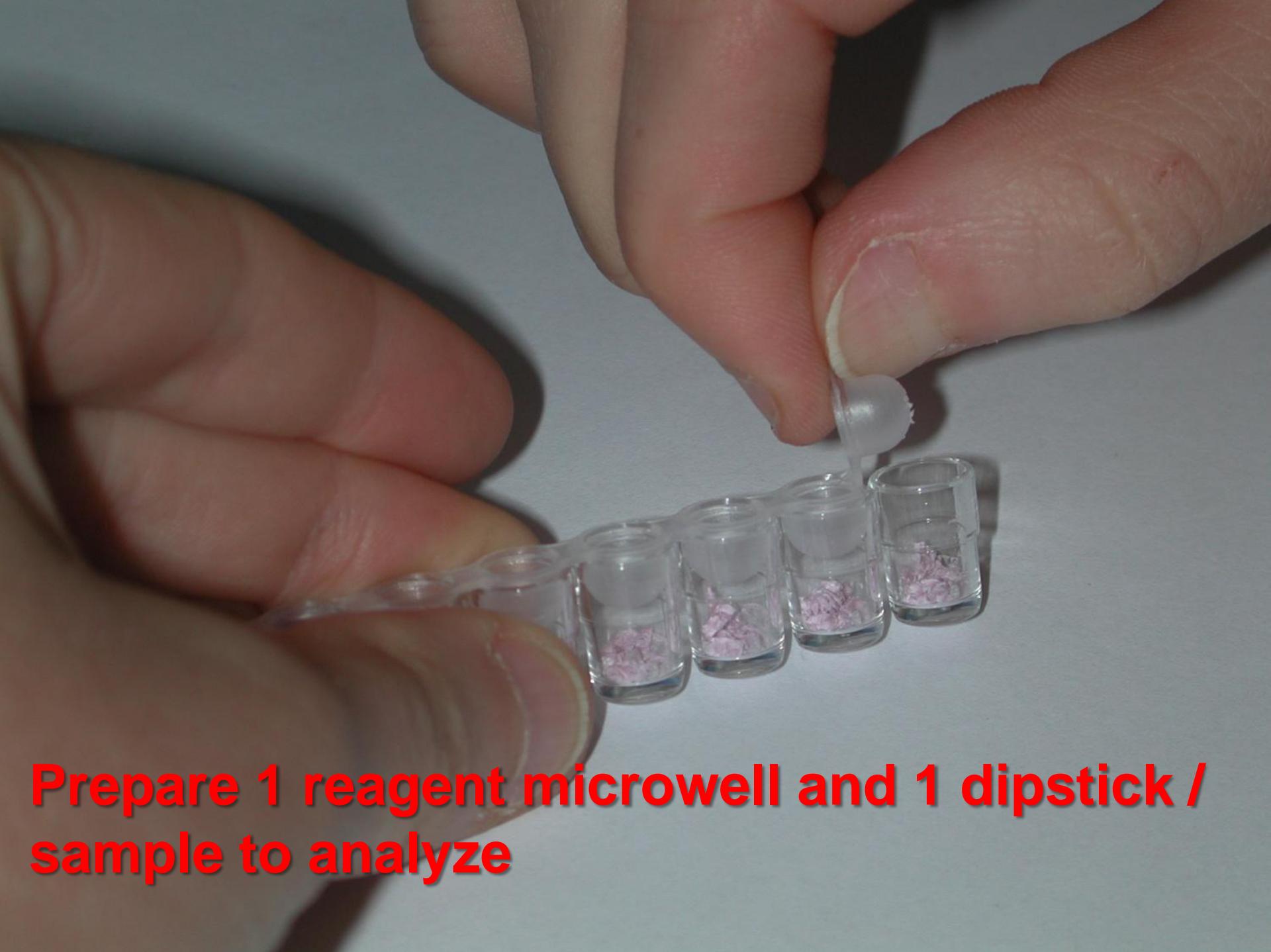
**Adjust both tubes (« A » and « B ») with
Dipstick Buffer to the mark (3.5 ml final
volume)**



**Combine and mix both samples
« A » and « B » (1:1)**

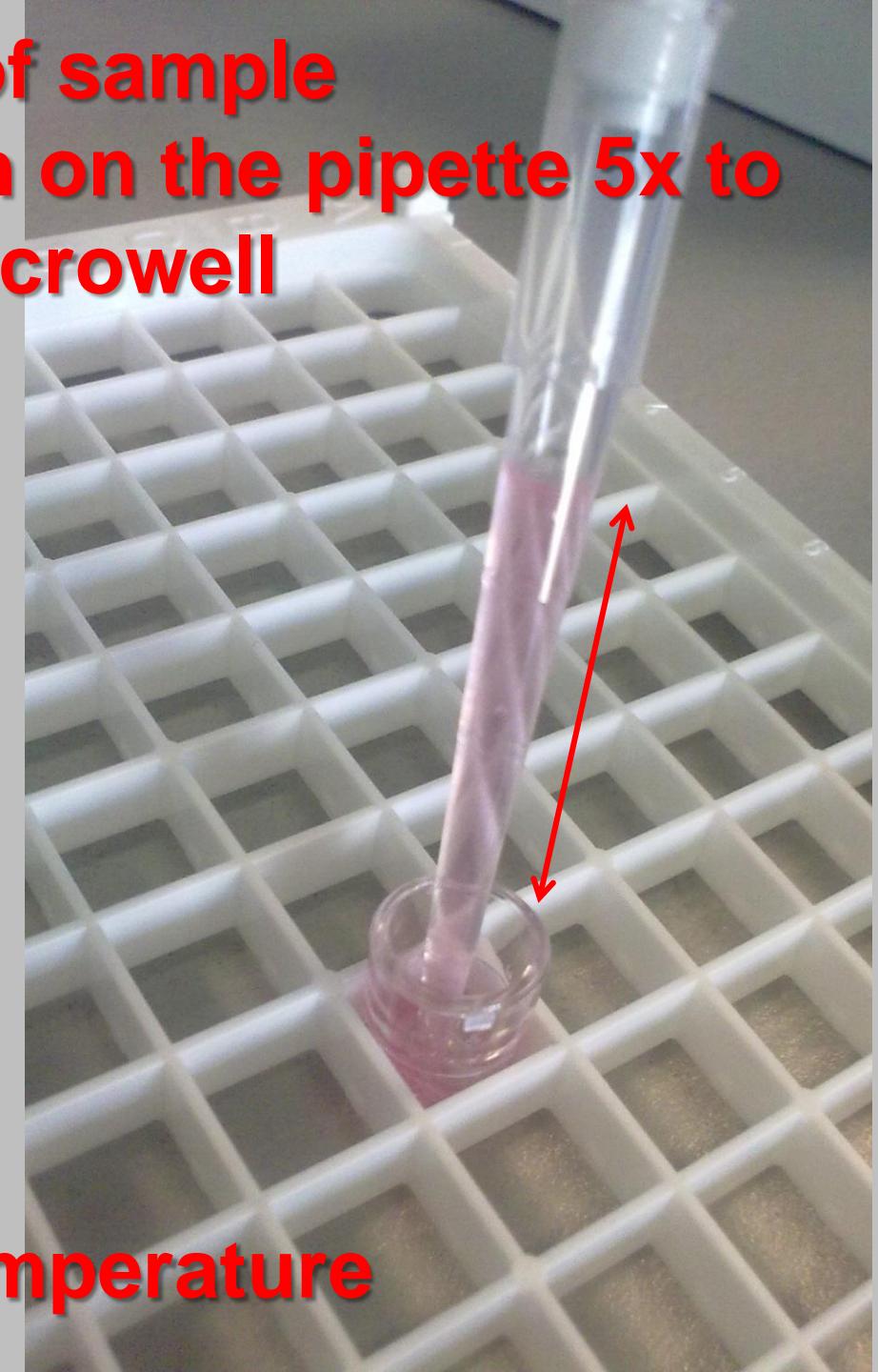
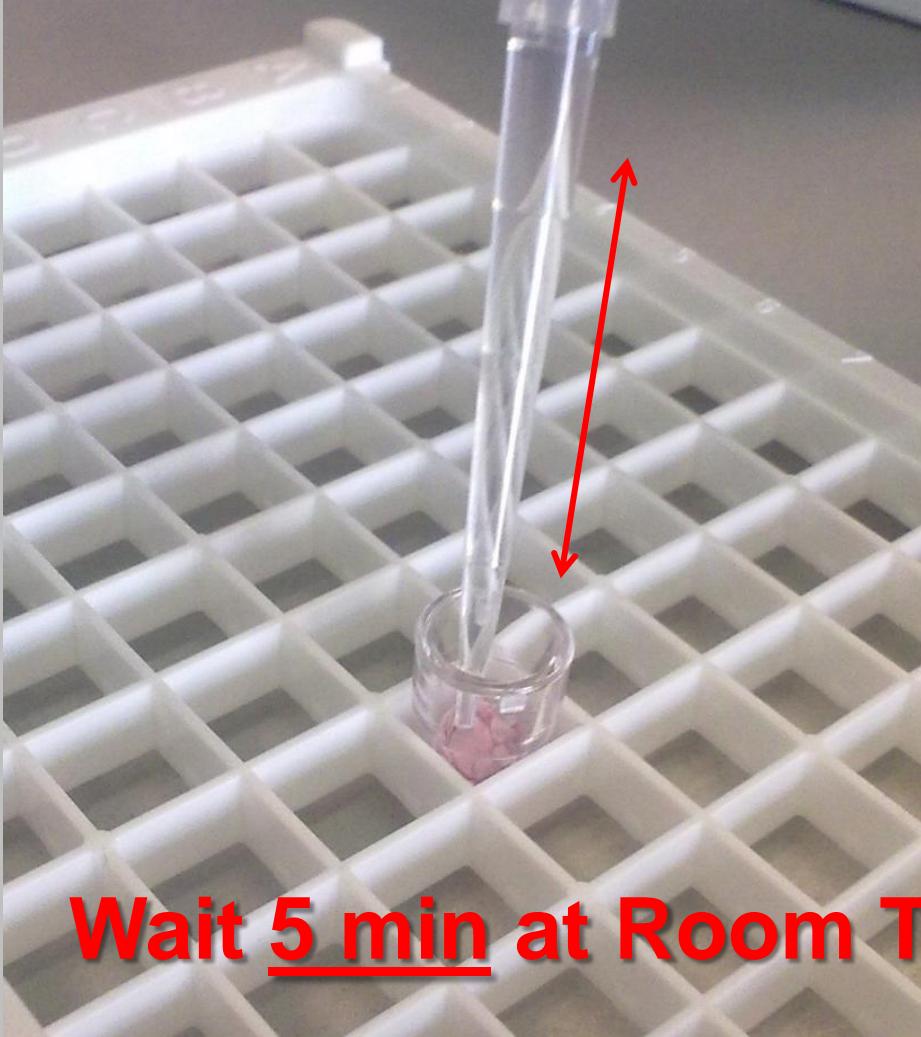


Open the dipstick tube

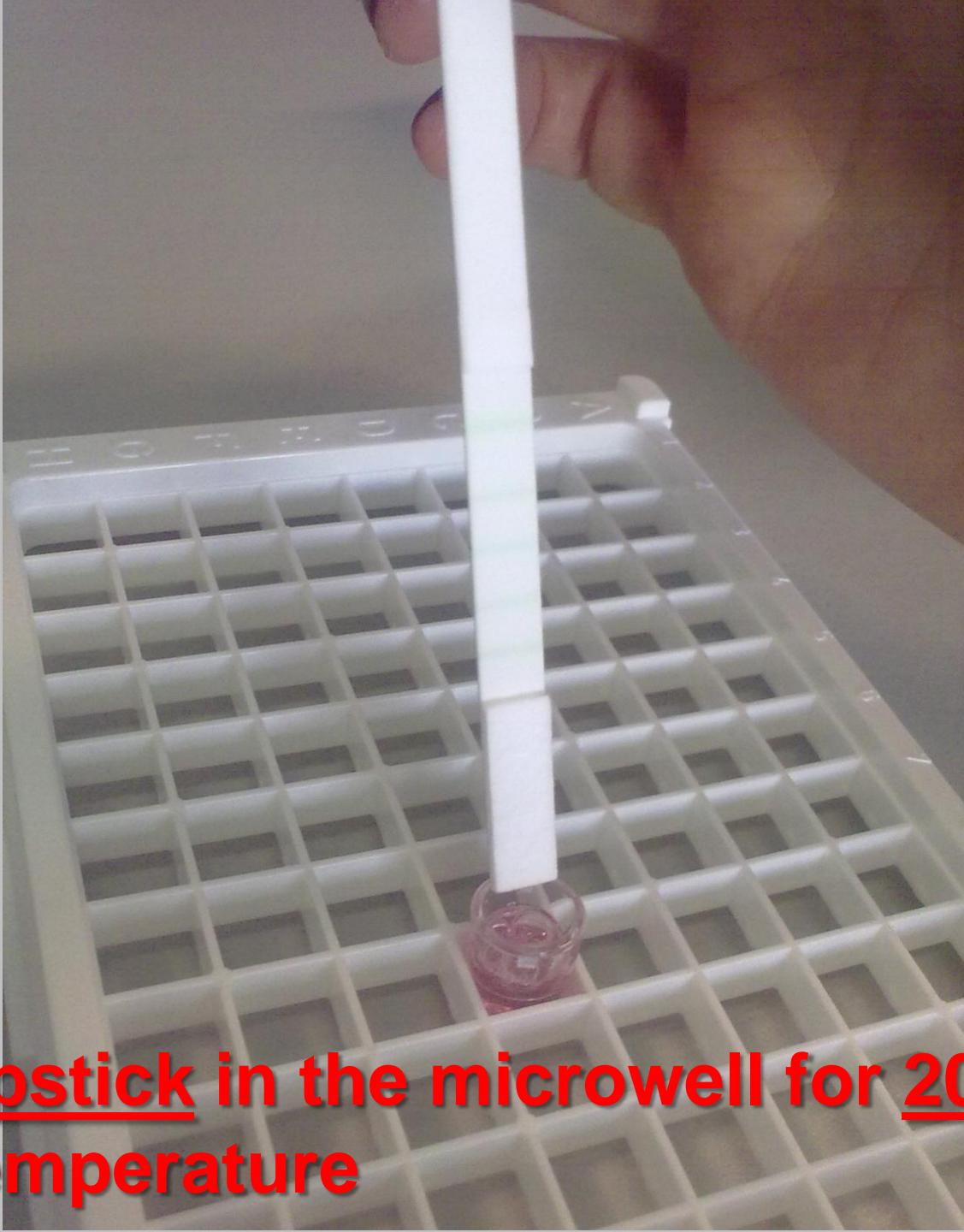


**Prepare 1 reagent microwell and 1 dipstick /
sample to analyze**

**Take 1 pipette (200 µl) of sample
(" mix A+B ") and push on the pipette 5x to
dissolve the reagent microwell**

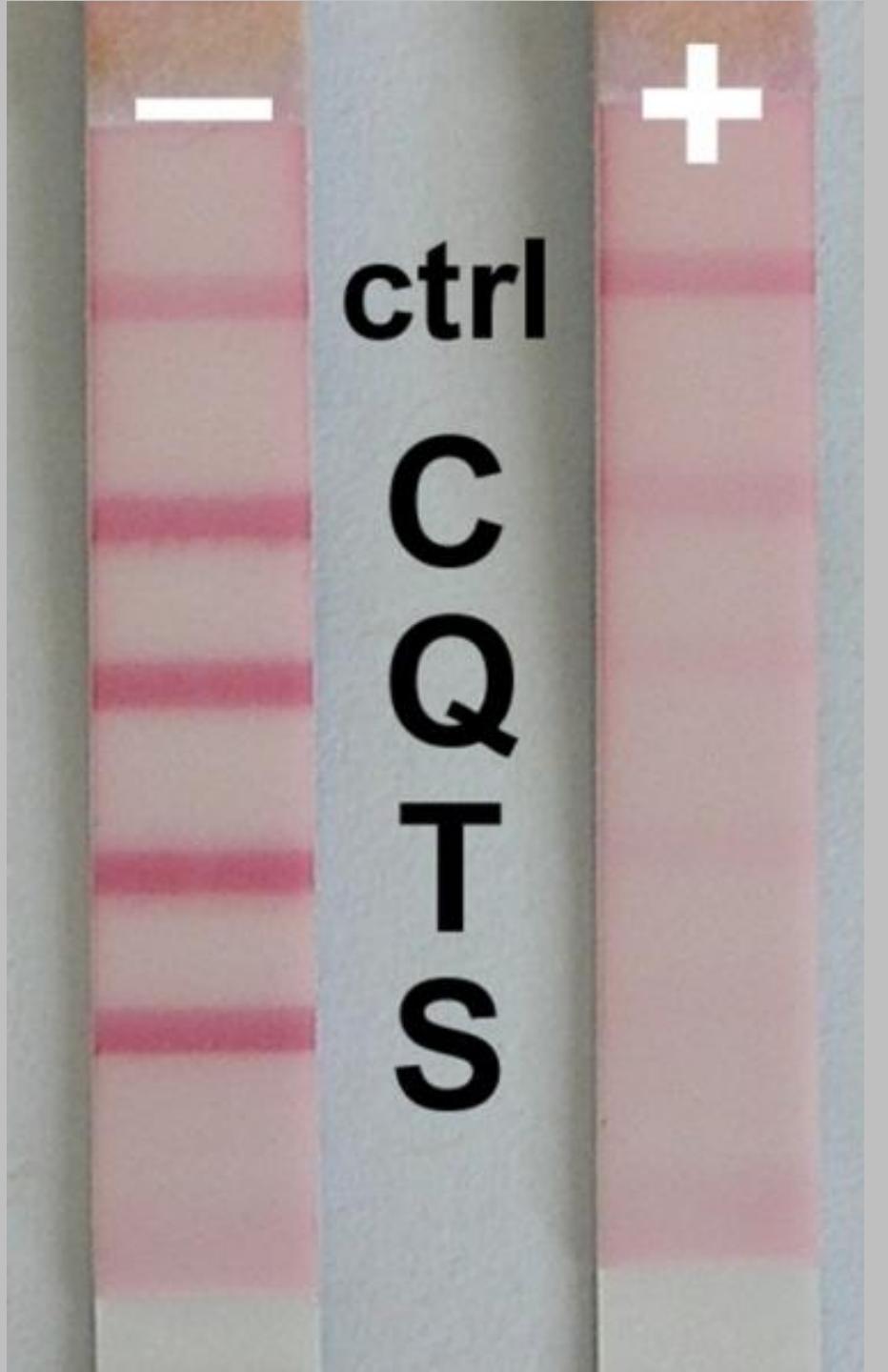


Wait 5 min at Room Temperature

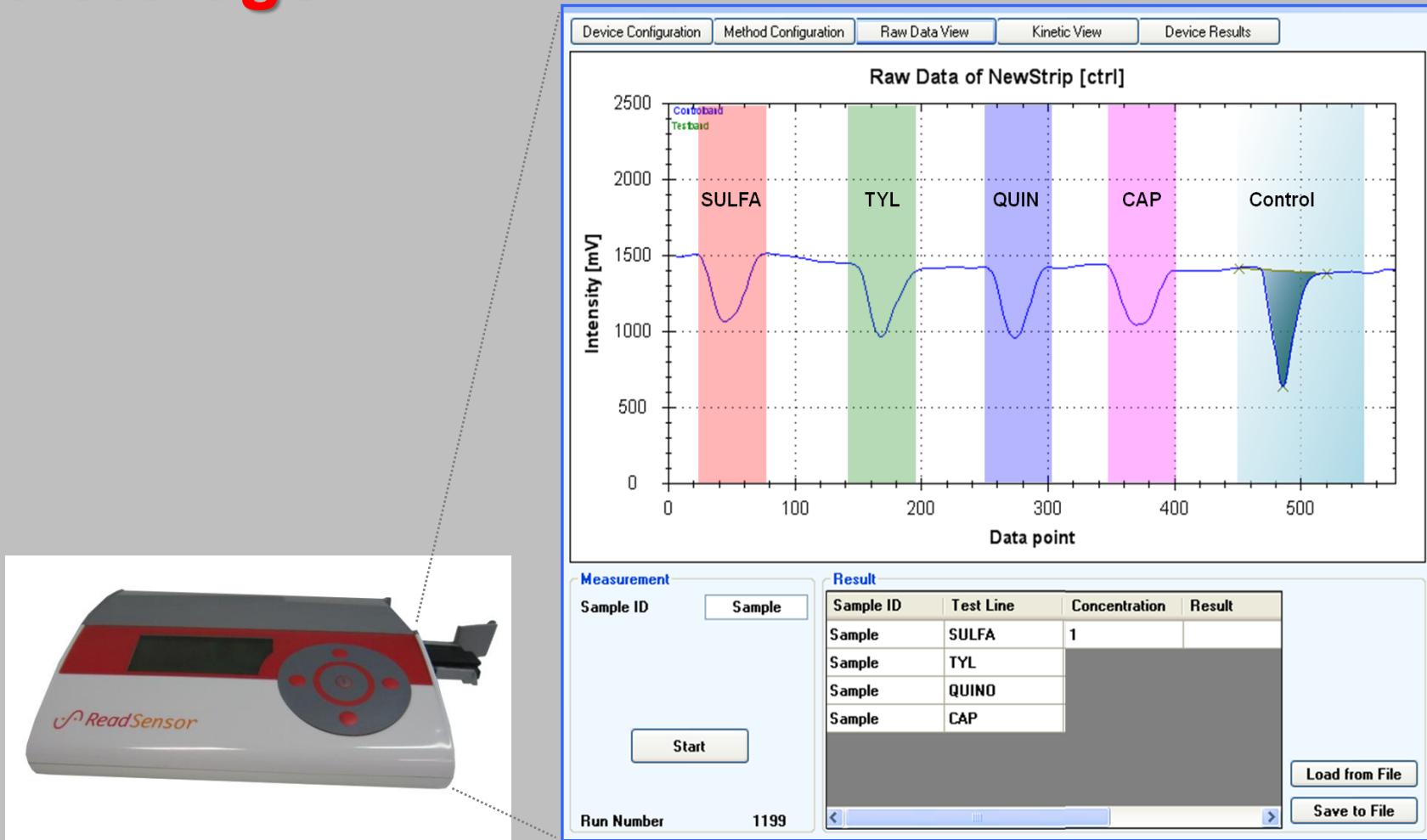


Add 1 dipstick in the microwell for 20 min at
Room Temperature

Remove the filter and compare the test lines with the control line



Optional : you can read the dipstick with the Readsensor® for confirmation of results and data storage



Expected sensitivity... pending validation

Sulfonamide compounds	LoD LAB	LoD FIELD	CRL**	(Fluoro)quinolone compounds	LoD LAB	LoD FIELD	CRL**
Sulfapyridine	<10	<50	50	Enrofloxacin	<25	<25	50
Sulfamethazine	<25	<50		Ciprofloxacin	<25	25	
Sulfamethoxypyridazine	25	50		Danofloxacin	25-50	<100	
Sulfamerazine	25	50		Difloxacin	250	<500	
Sulfamonometroxine	25	50		Marbofloxacin	50	<100	
Sulfadiazine	25	50		Norfloxacin	25	50	
Sulfadimethoxine	25	50		Sarafloxacin	>500	-	
Sulfathiazole	25	50		Flumequine	>500	-	
Sulfachloropyridazine	25	50		Other compounds	LoD LAB	LoD FIELD	CRL**
Sulfaquinoxaline	50	<200		Tylosin-A	10	10-50	10
				Chloramphenicol	5	<60	0.3

** European limits or recommended concentrations in honey (CRL – AFSSA-LMV France – SANCO /2006/3228)