CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Multiplex lateral flow immunoassays for the detection of pyrrolizidine, tropane and ergot alkaloids

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I) Introduction

Alkaloids are toxic secondary metabolites usually produced by plants. They contain one or more basic nitrogen atoms, usually located in a heterocyclic ring. They have important biological effects on the human health and can cause severe health problems. They are recognized as hepatotoxic, carcinogenic, teratogenic and mutagenic compounds. There are over 3000 compounds known such as caffeine, cocaine, codeine, morphine, nicotine and these may be classified into 14 different families according to the chemical structures of their ring. Alkaloids can contaminate feed and grains through botanical impurities, mostly with alkaloid-containing weeds, but also honey with contaminated pollens transferred by bees into honey. Facing these contamination problems of food, the european commission asked new developments for rapid tests for a better evaluation of risks related to specific alkaloids such as Pyrrolizidine (PA), tropane (TA) and ergot alkaloids (EA). In the framework of the Conffidence project (FP7), rapid lateral flow immunoassays are in development. The targeted toxins are jacobine and lycopsamine at 50 µg/kg in honey and feed for PA, atropine and scopolamine at 100 µg/kg in feed for TA and, ergotamine and ergocristine at 200 µg/kg in cereal and feed for EA.

II) Antibodies production

Antibodies raised against jacobine, lycopsamine, atropine, scopolamine, ergotamine and ergocristine have been produced by injecting to rabbits, alkaloids conjugated to BSA and BTG proteins (Figure 1).

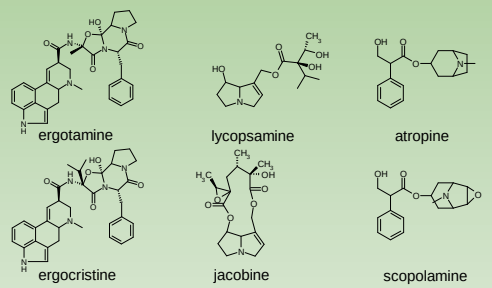


Figure 1 : Alkaloids that have been conjugated to BSA and BTG and used as immunogens

The IC_{50} of the best polyclonal antibodies and cross-reactivity profiles have been determined by ELISA and listed in Table 1.

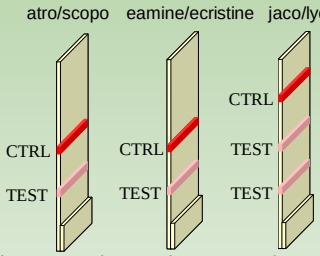
ELISA	ergotamine	scopolamine	jacobine	lycopsamine
IC_{50} (ng/ml)	76	0.4	0.5	21.4
Alkaloids	Cross-reactivity (%)			
atropine	-	**71**	-	-
scopolamine	-	**100**	-	-
jacobine	-	-	**100**	**6**
lycopsamine	-	-	**-**	**100**
senecionine	-	-	700	-
seneciphylline	-	-	350	-
retrorsine	-	-	700	-
erucifoline	-	-	108	-
echimidine	-	-	26	-
intermedine	-	-	-	273
ergotamine	**100**	-	-	-
ergocristine	**70**	-	-	-
ergosine	5	-	-	-

Table 1 : IC_{50} and cross-reactivity profiles of the produced antibodies

Due to the generic character of the antibodies, single test line dipstick assay have been produced for EA and TA while, for PA, a double test line dipstick assay has been considered.

III) Multiplex dipstick design

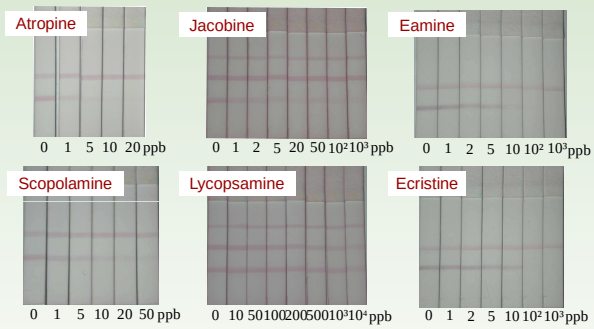
The dipsticks (Figure 2) have been produced by immobilizing a protein conjugate of scopolamine and ergotamine for the single test line TA and EA dipsticks. Regarding the PA dipstick, it was designed with two test lines due to the specific character of the antibodies raised against jacobine and lycopsamine. Dynamic control lines were used for defining positive and negative results.



Protocol of the test in buffer

1. Tip 200 µl of the contaminated buffer and mix until homogeneity
2. incubate 5 minutes at RT
3. Add the strips and let flow during 10 minutes
4. Interpret the final result with ReadSensor

Figure 2 : Scheme of TA, EA and PA dipsticks



The IC_{50} observed with dipsticks have been evaluated at 1 and 5 ng/ml for atropine and scopolamine with the TA dipsticks, at 5 and 10 ng/ml for ergotamine and ergocristine for EA dipsticks and at 1 and 500 ng/ml for PA dispticks.

IV) Conclusion

We have described in this work the first steps of development of multiplex dipstick tests for the detection EA, TA and PA in cereals, honey and feed. The results show that efficient antibodies have been produced against each alkaloid. With those antibodies, lateral flow immunoassays have been designed and show very satisfying sensitivities for each targeted alkaloids except for lycopsamine where an IC_{50} of 500 ppb has been determined. The next step of this work will be the studies on the recovery of alkaloids from real matrices and finally the adaptation of dipsticks from buffer to real samples.

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