PESTICIDES	REGULATORY MRL	LODS
INSECTICIDES – ORGANOPHOSPHATE GROUP		
Chlorpyrifos a ,j	10ppb	1ppb
Chlorpyrifos -methyl a	10ppb	1ppb
Diazinon a , h	20ppb	1ppb
Malaxon a	10ppb	10ppb
Paraxon -ethyl a ,j	20ppb	10ppb
Paraxon -methyl a, j	20ppb	1ppb
Phorate a , h , i	50ppb	10ppb
Malathion a , h, i	20ppb	10ppb
Fenithrothion a	10ppb	10ppb
Tetrachlorvinophos a	50ppb	1ppb
Monochrotophos a	20ppb	1ppb
Profenofos a	10ppb	10ppb
Dimethoate a, i	20-2000ppb	1ppb
Primiphos Methyl b , j	500ppb	10ppb
INSECTICIDE		
Abamectine a	10-30ppb	10ppb
Dinotefuron j	100 ppb	50 ppb
Ethion j	50 ppb	50 ppb
Fenpropathrin j	100 ppb	50 ppb
Flubendiamide j	100 ppb	01 ppb
Indoxacarb j	100 ppb	50 ppb
Chlorantraniliprole j	50 ppb	10 ppb
Thiamethoxam j	50 ppb	10 ppb
INSECTICIDES - CARBAMATE GROUP		
Bendiocarb a	50ppb	10ppb
Carbaryl a, c, d, h , j	50ppb	50ppb
Methomyl a, j	20ppb	1ppb
Propoxur a	50ppb	1ppb

PESTICIDES	REGULATORY MRL	LODS
Aldrin a, h, I , j	150ppb	10ppb
Dieldrin a , h, I , j	150ppb	100ppb
Endosulfan a , I , j	50ppb	10ppb
Heptachlor a ,h	150ppb	250ppb
FUNGICIDES		
Amisulbrom a	10 ppb	50ppb
Ametoctradin a	30 ppb	50ppb
Edifenphos a , c , j	10 ppb	10ppb
Ziram a	50 ppb	50ppb
Thiram a	50 ppb	50ppb
Maneb a	50 ppb	1ppb
Zineb a	10 ppb	1ppb
Captan a , c	20 ppb	0.1ppb
Iprodione c , f	10 ppm	1ppb
Benomyl j	100 ppb	50 ppb
Bitertanol j	50 ppb	10 ppb
Chlorothalonil j	100 ppb	100 ppb
Pyraclostrobin j	30 ppb	10 ppb
Thiophanate -Methyl j	100 ppb	50 ppb
HERBICIDES		
O-phenyl phenol	-	1ppb
Asulam a , j	50ppb	1ppb
Diuron d , f	500ppb	10ppb
Mesosulfuron methyl b , j	10ppb	1ppb
IMIDAZOLE		
Imazalil g	20ppb	10ppb
INSECTICIDE PYRETHROID ESTER		
Deltametrin c , a	2-5ppm	1ppb

a: Milk, h: Food Grains, i: Fruits, j: Fruit Juice, b: Wheat, c: Rice, d: Maize, f & n: Fruit & Nuts

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Patent Redg. No. 2213/DEL/2014

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SCOPE OF APPLICATION

Wide scope of application to screen water, processed fruit juices and cereal based foods for pesticide residues.

Surveillance/risk assessment in organized processed food industries

NOVEL FEATURES

- The developed paper strip assay is based on novel approach of exploiting spores as bio-recognition molecule as a source of marker enzyme (s) in prokaryotic system which other wise is used from eukaryotic system.
- No need of purification of enzyme and it's functional working has been established at 4 oC upto 8 months of storage.
- Paper strip assay detects Organo-phosphorous group (1-10 ppb), Carbamate group (1-50 ppb), organochlorines (10-250 ppb) and fungicide / herbicide (0.1-50 ppb) invariably within regulatory limits.
- Extraction protocol has been optimized successfully employing novel alternatives.
- The developed assay is cost effective, robust, reproducible, sensitive, selective and giving result in real time compared to conventional chromatographic techniques
- The overall assay is working within~2 hour which includes extraction of pesticides and its subsequent detection using paper strip assay.

METHODOLOGY FOR EXTRACTION OF PESTICIDE

Extraction of pesticide from feed & fodder samples:

Pesticide are extracted from feed & fodder sample as per following protocol:

Step-1: Mix equal quantity of reconstituted homogenise sample and solution 2, vortex and centrifuge @ 10000 rpm for 5 min at 37°C.

Step-2: Mix supernatant with clean up reagent (I), vortex and centrifuge @ 10000 rpm for 5 min at 37°C.

Step-3: Transfer solvent layer carefully to a tube containing cleanup reagent (II), vortex and centrifuge @ 10000 rpm for 5 min at 37°C.

Step-4: Separate out upper organic solvent layer and filter through specific filter tips.

Step-5: Evaporate filtrate using block heater at 80°C for 40 min.

The tube containing pesticide residue (Tube-2) is used to carry out paper-strip assay.

PAPER-STRIP PROTOCOL

Step-1-Reconstitution of lyophilized spores: Add 30 µL of buffer to reconstitute lyophilized spores (Tube-1)

Step-2-Enzyme pesticide interaction: Transfer reconstituted spores from Tube-1 to Tube-2 containing evaporated pesticide residues from extracts of spiked / feed & fodder sample and incubate in dry block heater at 37 °C for 40 min and vortex for 25 sec.

Step-3-Addition of paper strip: Add paper-strip functionalized with chromogen to test and control tube and incubate in dry block heater at 37°C for 20 min (Fig.2). After incubation, paper strips are air dried for 5 min after color development in control tubes.

Result interpretation: Development of sky blue color on paper strip, indicates absence of pesticide and no blue color indicates presence of pesticide (Fig3).

Fig. 3 : Result interpretation

Paper strip for rapid detection of Pesticide residues in animal feed & fodder

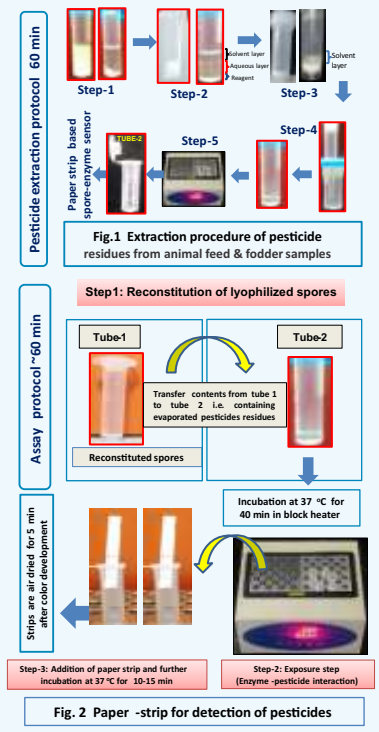
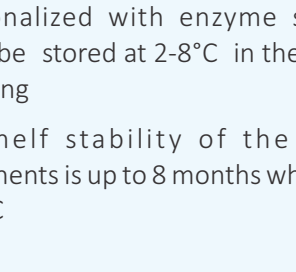


Fig.1 Extraction procedure of pesticide residues from animal feed & fodder samples

Fig. 2 Paper -strip for detection of pesticides

KIT PROTOTYPE COMPONENTS

Components used in extraction protocol



Cleanup reagents 1 & 2

Paper strip components

Lyophilized Spores

Functionalized Paper strips (vacuum packed)

Storage of kit components and their shelf stability

Lyophilized spores: The tubes containing lyophilized spores always be stored at 2-8°C

Vacuum packed strips: The strips functionalized with enzyme substrate always be stored at 2-8°C in their original packaging

The shelf stability of the test kit components is up to 8 months when stored at 2-8°C