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

PESTICIDES	REGULATORY MRL	LODS		
Aldrin a	150ppb	10ppb		
Dieldrin a	150ppb	100ppb		
Endosulfan a	50ppb	10ppb		
Heptachlor a	150ppb	250ppb		
	FUNGICIDES			
Amisulbrom a	10 ppb	50ppb		
Ametoctradin a	30 ppb	50ppb		
Edifenphos a	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	20 ppb	0.1ppb		
Iprodione c	10 ppm	1ppb		
Benomyl	100 ppb	50 ppb		
Bitertanol	50 ppb	10 ppb		
Chlorothalonil	100 ppb	100 ppb		
Pyraclostrobin	30 ppb	10 ppb		
Thiophanate - Methyl	100 ppb	50 ppb		
HERBICIDES				
O-phenyl phenol	-	1ppb		
Asulam a	50ppb	1ppb		
Diuron	500ppb	10ppb		
Mesosulfuron methyl	10ppb	1ppb		
	IMIDAZOLE			
Imazalil	20ppb	10ppb		
INSECTICIDE PYRETHROID ESTER				
Deltametrin a	2-5ppm	1ppb		

a: Milk



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Technology Developed By National Referral Centre, ICAR-National Dairy Research Institute, Karnal Patent Redg. No. 2213/DEL/2014



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

Surveillance/risk assessment in organized dairy farms and 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.
- In current approach there is 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 demonstrated LODs for 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 novelalternatives.
- 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 EXTRACTION OF PESTICIDE FROM MILK

Extraction of pesticide from milk: Pesticide are extracted from spiked reconstituted skimmed milk (RSM) / natural milk sample as per following protocol:

Step-1: Mix equal quantity of milk sample and organic solvent, 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 μ L 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 / natural milk 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.3). 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 in milk (Fig1).

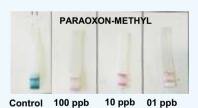
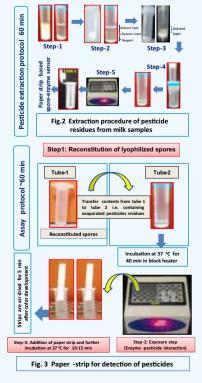


Fig. 1 : Result interpretation

Paper strip for rapid detection of Pesticide residues in milk





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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