



Two Stage Enzyme Assay for Detection of *L. Monocytogenes* in Milk

COBIA VALUE ADDED PRODUCTS

Technology works based on the principle of targeting "enzyme-substrate reaction for specific marker enzyme (s) to release free chromogen that can be visually detected by colour change" Assay can confirm the presence of *L. monocytogenes* within in real time of 4.30±0.10h after initial pre-enrichment of milk samples in novel selective medium i.e. LSEM for 18h or 24h Appearance of black color indicates the presumptive presence of *Listeria* spp. within the given specified time limits i.e. 24±2hrs at 1.4 log cfu levels for 25g/ or 23.15±1.0hrs per g of the milk sample. The appearance of Green colour indicates the confirmation of *L. monocytogenes* within specified time limits of 4.30±0.15hrs in lyophilized Enzyme substrates mixture/ or in 2.30±0.15hrs in Liquid form
Technology has been validated with raw milk, pasteurised milk, ice

Name Of institute: NDRI, Karnal
Stage of development:
Ready for commercialization
Patent status: Filed

Scientific Experts:
Dr.Naresh Kumar,

Background

L. monocytogenes is well known to cause human listeriosis with high rate of mortality up to 30%. The outbreaks are associated with the consumption of raw milk, pasteurized milk, ice cream and different varieties of cheese causing potential health concern to human beings. In recent development FSSAI has recommended to screen all milk and milk products for *L. monocytogenes* as regulatory compliance to ensure safety of consumers. In view of food safety concern and its regulatory requirements the new technology developed at NDRI has

Benefits / Utility

Effective monitoring of high-risk dairy and other food products for surveillance and safe food delivery Release of food products within one working day to meet the market demand and supply while also ensuring consumers safety Cost effective technology when compared with existing prior art Technology can be used as assay

Country Context

The prevalence of *L.monocytogenes* is more in developed countries. Regulatory compliance in the national regulation, The FSS Act 2011is mandatory.

Scalability

The technology is suitable for small and medium enterprises that can transform the process into a kit prototype for industrial application as well as can also be adopted by the dairy / food industry for regulatory compliance [without added cost!!!].

Business and Commercial Potential

Moderate business and commercial potential is significant in view of global regulatory requirements on *Listeria monocytogenes* in food products.

Potential investors to this technical innovation

Stake holders with have business in diagnostic kits and dehydrated media preparation can adopt this technology without added cost in their existing facility.



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Financials

Total Capital Investment(excluding Land and licensing fees) : Rs 20-25 lakhs Break up of capital investment: in routine microbiological facility required for aseptic work which includes biosafety cabinet, centrifuge, autoclave, pH meter, Electronic balance, Incubator, Auto pipettes, vacuum drying system etc Variable cost (per /unit): Rs 75/- Break of variable cost: consumables, dehydrated media, selective agent, enzyme substrates etc. Expected sale/unit: Appr. 1000 test kit per month in the beginning and may go up significantly if assay

Target Market / Customer

1. Dairy industry 2. Milk processing industry 3. Pharmaceutical units
4. FSSAI approved laboratory 5. NABL accredited laboratory
6. R & D independent test houses

Limiting factors for large scale commercialization

Modus operandi to get clearance for product trials from stake holders before licensing / MOU agreement

Social impact of the technology

L. Monocytogenes is well known to cause human listeriosis with high rate of mortality up to 30%. The outbreaks are associated with the consumption of raw milk, pasteurized milk, ice cream and different varieties of cheese causing potential health concern to human beings. Recently, FSSAI has implemented a "zero tolerance" policy for monitoring *L. monocytogenes* in dairy products Enzyme-substrate assay using chromogenic and fluorogenic substrate (s) have found greater applicability in prior art for detection of high risk food borne pathogens in clinical samples, meat and dairy products, as they are rapid, sensitive and less time-consuming
Any other relevant information

Spore germination based assay for *L. monocytogenes* has been developed on chip surface and its optimization on micro well chip is under progress for its integration with multi-analyte sensor development in collaboration with other consortia partners. Spore based concept is not within the scope of this technology.