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Rapid Detection technic for food bornpathogens

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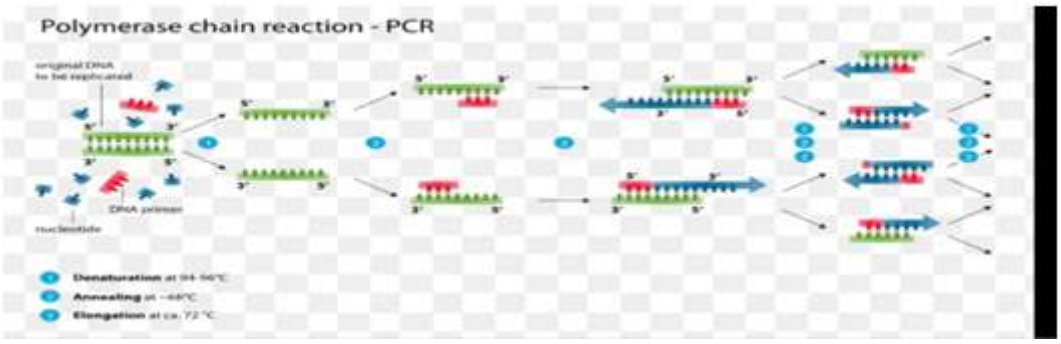
ABSTRACT
Food safety is increasingly becoming an important public health issue, as foodborne disease present a widespread and growing public health problem in both developed and developing countries. The rapid and precise monitoring and detection of foodborne pathogens are some of the most effective ways to control and prevent human foodborne infections. Traditional microbiological detection and identification methods for foodborne pathogens are well known to be time consuming and laborious as they are increasingly being perceived as insufficient tomeet the demands of rapid food testing. Recently, various kinds of rapid detection,identification, and monitoring methods have been developed for foodborne pathogens,including nucleic-acid-based methods, immunological methods, and biosensor- based methods, etc. This article reviews the principles, characteristics, and applications of recent technicand food born pathogen.

Keywords: Rapid Detection Technic for food born pathogen, different techniques for food born pathogens,real-time PCR.

I. INTRODUCTION

1.The occurrence of foodborne diseases has increased over the years which resultin major public health problem worldwide. Foodborne pathogens can be found invarious foods .So it is important to detect foodborne pathogens to provide safefood supply .To prevent foodborne diseases, the conventional methods used todetectfoodbornepathogenaretimeconsumingandlaborious.Hence,avarietyofmethod have been developed for rapid detection of foodborne pathogens and itis required in many food analyses. Rapid detection methods can be categorizedintonucleicacid-based,biosensor-basedandimmunological-basedmethods.Thisarticle emphasizes on the principles and application of recent rapid methods forthe detection of foodborne bacterial pathogens. Detection methods included aresimple polymerase chain reaction (PCR), multiplex PCR, real-time PCR, nucleicacid sequence-based amplification (NASBA), loop-mediated isothermalamplification (LAMP) and oligonucleotide DNA microarray which classified asnucleicacid-basedmethods;optical,electrochemicalandmass-basedbiosensorswhich classified as biosensor-based methods; enzyme-linked immunosorbentassay (ELISA) and lateral flowimmunoassay which classified as immunological-based methods. In general, rapid detection methods are generally time-efficient,sensitive,specificandlabor-saving.Thedevelopmentsofrapiddetectionmethodsarevitalinpreventionandtreatmentoffoodbornediseases .Soit'shelpustosavelife to being in danger like in case of covid-19.Its very difficult to recover withdisease (many things are involve like vaccines,drugs,herd immunity,economy,population etc.)As we know drug and vaccine making a laborious and timeconsumingprocess.



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Techniquefordetectionoffoodbornpathogens:-

Theconventionalmethodsfordetectingthefoodbornebacterialpathogenspresentinwhichfoodarebasedonculturingthemicroorganismsonagarplatesfollowedbystandardbiochemicalprocess.Conventional methodsareusuallyinexpensiveandsimplebutthesemethodscanbetimeconsumingastheydependontheabilityofthemicroorganismstogrowindifferentculturemediasuchaspre-enrichmentmedia,selectiveenrichmentmediaandselectiveplatingmedia.Usuallyconventionalmethodsrequire2to3daysforpreliminaryidentificationandmorethanaweekforconfirmationofthespeciesofthepathogens.Recently,differentrapidmethodswithhighsensitivityandspecificityhavebeendevelopedtoovercometheli mitationsofconventionalmethodsforthedetectionand identificationoffoodbornepathogens.Rapiddetection methodsareimportant,particularlyinfoodindustry,ast heyareabletodetectthepresenceofpathogensinrawan dprocessedfoodsinlesstime.Rapidmethodsarelessti meconsuming,labor-savingandabletoreducehumanerrors.Thesearefollowing:-.1Nucleic-acid-basedmethods2.Immunologicalmethods3.Biosensor-basedmethods.

1.Nucleicacid-basedmethods:-

Nucleicacid-basedmethodsoperatebydetectingspecificDNAorRNAsequencesinthetargetpathogen.Thisisdonebyhybridizingthetargetnucleicacidsequencetoasyntheticoligonucleotide(probesorprimers)whichiscomplementarytothetargetsequence.Thereareusedinmanybacterialpathogenssuchas Clostridium botulinum,Vibriocholerae,Staphylococcusaureus.These are of following types:-

a.SimplePCRMethod

PCR have been used in the detection of numerous foodborne pathogens like Listeria monocytogenes, Escherichia coli O157:H7, Staphylococcus aureus, Campylobacter jejuni,Salmonellaspp.andShigellaspp.

In this method, double-stranded DNA is denatured into single strands (94°C), and afterthatannealingwithprimers(68°C)whichfollowedbyextensionoftheprimerscomplementarytothesingle-strandedDNA(72°C)andwithathermostableDNApolymerase.Thesestepsarerevised,resultingindoublingoftheinitialnumberoftargetsequenceswitheachcycle.Ethidium-bromide-stainedusedasadyeinelectrophoresisgelfor visualisingthe Source:Bolton et al (2000).

b. Multiplex PCR

1.Chenetal. (2012)had carriedoutmPCRforthesimultaneousdetection.MultiplexPCR(mPCR)istheprocessin which several specific primer sets are combined into a single PCR assay . Thedesigntheprimersisakeyfactorinthedevelopmentofamultiplex PCRassay.There may be some interaction between the multiple primer sets, so the primerconcentrations may have to be adjusted in order to generate reliable yields of allthe PCR products. Meanwhile, the primer sets should be designed with a similarannealingtemperature.
Source:Bolton et al(2000).
Source: Bolton et al (2000).

c.QuantitativePCR

1.Quantitative PCR (qPCR), also called real-time PCR, is aprocess capable ofcontinuouslymonitoringthePCRproductformation throughoutthereaction.Itisrapidlybeingappliedinfoodmicrobiology.Usingthismethodweallowsquantifying one specific microorganism in food and studying its behavior as aconsequenceoftheinfluenceoftheenvironment(i.e.,foodcomposition,temperature,pH,oxygen,etc.)bystudyingexpressionofsuitabletargetgenes.Afterthisprocessnoneedforpost-amplificationtreatmentofthesamples,suchasgelelectrophoresis,soreducingthetimeofanalysis.

d.Nucleicacidsequence-basedamplification(NASBA)

NASBAisnormallyusedfortheamplification of RNA,thenthe single-stranded RNA template is converted intocomplementaryDNA(cDNA)bythereversetranscriptaseduringthereaction.NASBAreactionoccursataround41°C.

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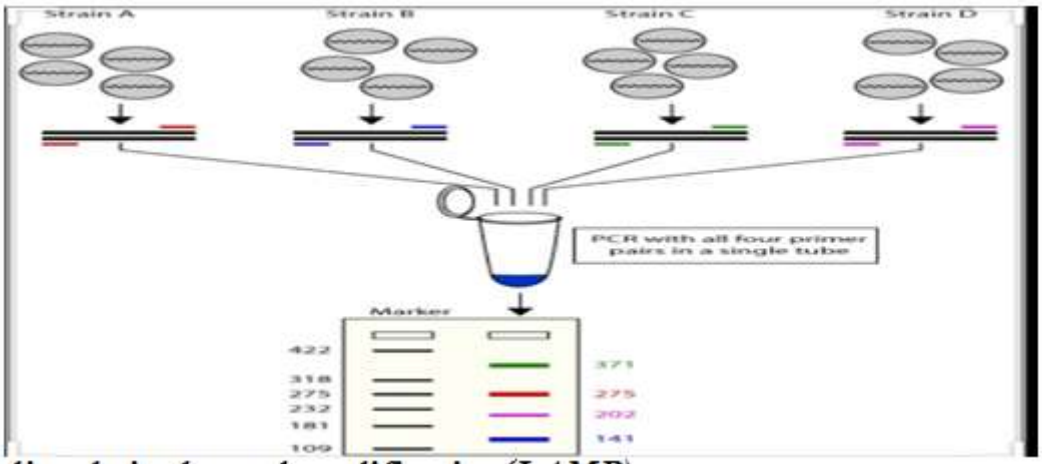
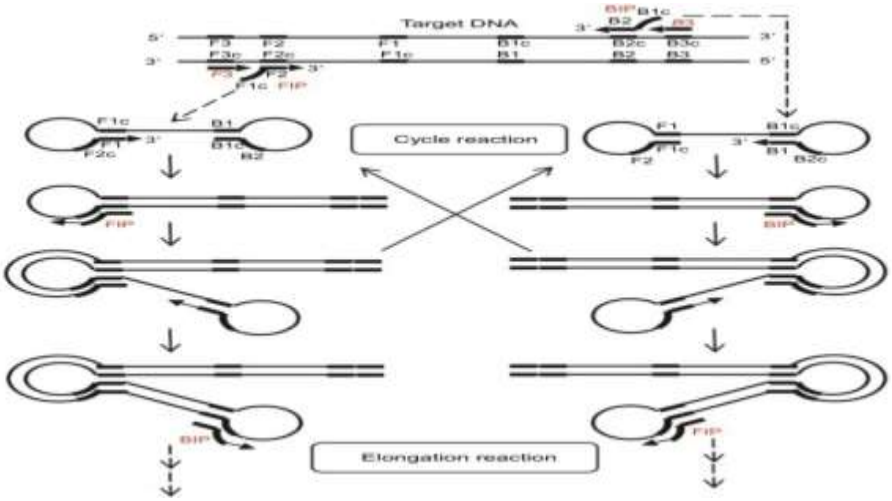


Loopmediated isothermalamplification(LAMP)

LAMPisbasedonauto-cyclingstranddisplacement DNA synthesis carried out by Bst DNA polymerase large fragmentunder isothermal conditions between 59°C and 65°C for 60 min. In LAMP, fourprimers comprising two inner primers and two outer primers are used to targetsix specific regions of target DNA



Source:Bolton et al(2000).

Biosensor-basedmethods

It is ananalyticaldevicethatconsistsoftwomainelements:a bioreceptor and a transducer. The bioreceptor responsible for recognizing thetargetanalytewhichcaneither bea:-

1 Biological**material:enzymes,antibodies,nucleicacidsandcellreceptors.**

2.Biologicallyderivedmaterial:aptamersandrecombinantantibodie3.Biomimic:imprintedpolymersandsyntheticcatalysts.

TheBiosensorscanbeoptical,electrochemical,mass-based,thermometric,micromechanical or magnetic. Biosensors are easy to operate and they do notrequiresamplepre-enrichment(unlikenucleic-acidbasedmethodsandimmunological methods which require sample pre-enrichment for concentratingthepathogens before detection.

a.Opticalbiosensor

1.The most commonly used optical biosensor for the detection of foodborne pathogenissurfaceplasmonresonance(SPR)biosensorbecauseoftheirsensitivity.SPRemploys reflectance spectroscopy for the pathogen detection. In SPR, bioreceptors areimmobilized on the surface of a thin metal

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b.**Electrochemical biosensors**

1.Electrochemicalbiosensorsarefurtherclassified into severaltypessuchas:-

Amperometric, impedimetric, potentiometric, and conductometric according tothe measurement of changes in current, impedance, voltage and conductancerespectively,whichcausedbyfoodbornpathogen-bioreceptorinteractions.

c.Mass-basedbiosensors

1. Mass-based or mass-sensitive biosensors operate based on the detection of smallchangesinmass.Mass-basedbiosensorsinvolvetheuseofpiezoelectriccrystalwhichwill vibrate at a certain frequency when induced by an electrical signal of a certainfrequency. The bioreceptors (e.g., antibodies) for the detection of food bornpathogens(e.g., antigens) are immobilized on this crystal. Once the target antigens bind to theantibodies immobilized on the crystal, this will cause a measurable change in thevibrationalfrequencyofthecrystalwhichcorrelateswiththeaddedmassonthecrystalsurface.

3.Immunological-BasedMethods

Thedetectionoffoodbornepathogensbyimmunological-basedmethodsisbasedonantibody-antigeninteractions,wherebyaparticularantibodywill bindtoits specific antigen. The binding strength of a particular antibody to its antigendeterminesthesensitivityandspecificity.Immunological-basedmethodsinvolvetheuseofpolyclonalandmonoclonalantibodies It is of following types:-

a.Enzyme-linkedimmunosorbentassay(ELISA)

1.ELISA is also commonly used for the detection of toxins present in foods suchas Clostridiumperfringens α, β, andεtoxin, staphylococcalenteroxins A, B, C, and E, botulinumtoxins and Escherichiacoli enterotoxins.

2.ELISAisoneofthemostcommonlyusedimmunologicalmethodsforthedetection of foodborne pathogens. Sandwich ELISA is the most effective form ofELISAwherebyitinvolvestwoantibodies.Theprimaryantibodyisusuallyimmobilized onto the walls of the microtiter plate wells.

The target antigen likebacterial cells or bacterial toxins from the food sample binds to the immobilizedprimaryantibodyandtheremainingunboundantigensarewashedout.Afterthat,anenzyme-conjugatedsecondaryantibodyisaddedanditwillbindtotheantigenandtheremainingunboundantibodiesare washedout.Thecomplexantigensandwichedbetween twoantibodiesisformedanditcan be detected by adding a colorless substrate which will be converted into acoloredforminthepresence oftheenzyme.

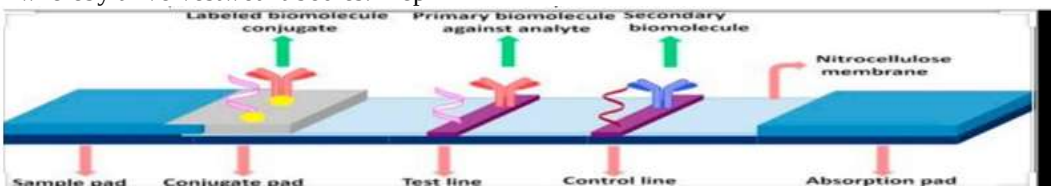
3.There are different types of enzymes can be used in ELISA, some of the mostcommonlyusedenzymesincludehorseradishperoxidase(HRP),alkalinephosphataseandbeta-galactosidasewhichproducedcolouredproduct.Thecolourproductisfurtherdetectedforpresenceorabsence offoodbornpathogens.

b.Laternalflowimmunoassay:-

1.Lateralflowimmonuassayisalsousedfordetectionof otherfoodbornebacterialpathogenssuchas Listeriaspp. and Salmonella(Kimetal.,2007;Shuklaetal., 2011)

2.It'sdeviceismadeupoffoursectionswhicharearrangedorderly on a plastic backing, with sample pad starting at the bottom, followed byconjugate pad, nitrocellulose membrane and then absorbent pad. The samplefluidwillmigratealongthefoursectionsoflateralflowimmunoassayviacapillary action. The sample fluid encounters and mixes with the conjugate,whichcanbeantibodyorantigenlabeledbyacolorparticle,attheconjugatepadandthenpassthrough thelinesinthenitrocellulosemembranethatimmobilizedwith antibody or antigen. The color particle can bind to the antibody or antigenimmobilized at test line depending on the analytes present in the sample.

Thecolorcanbevisualizedapproximatelytwoto10min aftertheadditionofsample.



Source: Zhao et al (2014).

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Theadvantageanddisadvantagearefollowing:

Detectionmethod	Advantages	Limitations	References
Nucleica cid based			
SimplePCR	*Highsensitivity *Highspecificity *Automated *Reliableresults	AffectedbyPCRinhibitors,Requires DNApurification. Difficulttodistinguishbetweenviableandnon-viablecells	Mandaletal.,2011;Zhang, 2013;Parket al.,2014
Multiplex PCR	Highspecificity *Automated *Reliableresults	AffectedbyPCRinhibitors Difficulttodistinguishbetweenviableandnon-viablecells	
	Highsensitivity	• Primerdesigniscrucial	
	Detection ofmultiplepathogens		
Real-timPCR	Highsensitivity	Highcost.	
	• Rapidcycling	Difficultformultiplexreal-timePCRassay	

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	Doesnotrequirepost-amplificationproductsprocessing	AffectedbyPCRinhibitors. Difficulttodistinguishbetweenviableandnon-viablecells. Requirestrainedpersonnel.	
	Real-timemonitoringPCRamplificationproducts		
NASBA	SensitiveSpecificLowcost Does notrequirethermalcyclingsystem. Able todetectviablemicroorganisms.	Requiresviablemicroorganisms.Difficultiesinhandling RNA.	LauriandMariani, 2009;Zhaoetal.,2014
LAMP	Allowsdetection of specific	Primerdesignisocomplicated.	Zhao et al., 2014
	serotype•Labor- saving.	Insufficienttodetectunknownor unsequencedtargets.	

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	Enablesdetection ofmultiplepathogens.		
Oligonucleotide DNA microarray	Highly sensitivity	Highcost.	
2.Biosensor-baseda.Opticalbiosensors	Highlyspecific. Enablesreal-time or near real-timedetection. Label-freedetectionsystem.	Highcost.90th	Ivnitskietal.,1999;Mandal et al., 2011; Zhang,2013
Electrochemicalbiosensors	Can handle largenumbers ofsamples. Label-free detection. Automated.	Notsuitableforanalyzingsampleswithlowamountofmicroorganisms. • Analysis mayinterferedbyfoodmatrices • Manywashingsteps Mass-basedbiosensors •	Ivnitskietal.,1999;Mandal et al., 2011; Zhang,2013

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	Costeffective.		
Mass-basedbiosensors	Easytooperate • Lowsensitivity • Label-freedetection ofbacteria	Longincubationtime. Manywashingand dryingsteps. • Regenerationofcrystalsurfacemaybe problematic	Ivnitskietal.,1999;Mandal et al., 2011; Zhang,2013
	• Real-timedetection		
Immunological-based *ELISA	• Specific • Can be automatedsothatitismore timeefficientandlabor-saving • Falsenegativeresults • Allowsthedetectionofbacterialtoxic. • Canhandlelarge numbersofsamples	• Mayresultincross-reactivitywithclosely relatedantigens. Pre-enrichmentisrequiredinordertoproducethecellsurfaceantigens. Falsenegativeresults. Requireslabelingofantibodiesorantigens.	Zhang,2013;Parketal.,2014;Zhaoet al.,2014

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Lateralflow Immuno	Low cost.Reliable	Requireslabelingof antibodiesorantigens.	Zhao et al.,2014
assay	• Easytooperate • Sensitive • Specific • Allow the detection ofbacterialtoxins.		

II. CONCLUSION:

Herearticle conclude that the rapid detection technique are more effective thanconventional method in thecircumstances ofdifferent food born pathogendisease.Becthesearecosteffective,lesstimeconsuming.Theseartheprimarily requirements for today's time.As we know the detection of food bornpathogenisgood steptowards living a disease free life. We know conventionaltech.are also their but the rapid detection are giving speed to work and costeffective. It includes nucleic acid-based methods such as NASBA and LAMP areavailable for the detection of foodborne pathogens and their toxins. NASBA andLAMP are relatively sensitive, specific and cost efficient. They do not requirethermocyclingsystemthereforetheyareuseful especiallyinlowresourcesettings. Also, numerous biosensors-based methods have recently emerged andemployedinthefieldoffoodbornepathogendetectionduetotheirrapidnessandcost effectiveness. Biosensors-based methods are easy to operate and they donotrequiretrainedpersonnel,furthermorethetechniquescanbeusedforthe detectionoffoodbornepathogenswithoutsamplepre-enrichment.But,improvementinfoodmatrixesdetectionisstillneededforthesemethodsforon-site detection. Immunological-based methods such as ELISA and lateral flowimmunoassay are also used for the detection of foodborne bacterial pathogensand their toxins. Immunological methods work best in the absence of interferingmoleculesinthesamplessuchasnon-targetedcells,DNAorproteins.Combinationofseveral rapidmethodsforthedetectionofaparticularfoodborne pathogen is also possible as the use of only one detection method may not besufficient to confirm the detected pathogen. Further studies on the effect ofdifferent combinations of rapid methods for foodborne pathogen detection arerequiredinordertodevelopthemosteffectiveandaccuratedetectionmethod.

REFERENCE:

[1]. 1.Bolton F. J., Fritz E., Poynton S. (2000). Rapid enzyme-linked immunoassay for the detection of Salmonella in food and feed products: performance testing program. J. AOAC Int. 83, 299–304. [PubMed] [Google Scholar

[2]. Call D. R., Brockman F. J., Chandler D. P. (2001). Detecting and genotyping Escherichia coli O157:H7 using multiplexed PCR and nucleic acid microarrays. Int. J. Food Microbiol. 67, 71–80. 10.1016/S0168-1605(01)00437-8 [PubMed] [CrossRef] [Google Scholar]

[3]. Carvalho R. N., Oliveira A. N., Mesquita A. J., Rezende C. S. M., Mesquita A. Q.,Romero R. A. M. (2014). PCR and ELISA (VIDAS ECO O157®) Escherichia coliO157:H7 identification in Minas Frescal cheese commercialized in Goiânia, GO.Braz.J.Microbiol.45, 7–10.10.1590/S1517-

[4]. Lauri A., Mariani P. O. (2009). Potentials and limitations of molecular diagnostic methods in food safety. Genes Nutr. 4, 1–12. 10.1007/s12263-008-0106-1 [PMC free article] [PubMed] [CrossRef] [Google Scholar

[5]. Mandal P. K., Biswas A. K., Choi K., Pal U. K. (2011). Methods for rapid detection of foodborne pathogens: an overview. Am. J. Food. Technol. 6, 87–102 10.3923/ajft.2011.87.102 [CrossRef] [Google Scholar] [Ref list]

[6]. Park S. H., Aydin M., Khatiwara A., Dolan M. C., Gilmore D. F., Bouldin J. L., et al. . (2014). Current and emerging technologies for rapid detection and characterization of Salmonella in poultry and poultry products. Food Microbiol. 38, 250–262. 10.1016/j.fm.2013.10.002 [PubMed] [CrossRef] [Google Scholar]

[7]. Mandal P. K., Biswas A. K., Choi K., Pal U. K. (2011). Methods for rapid detectionoffoodbornepathogens:anoverview. Am.J.Food.Technol.6,8710210.3923/ajft.2011.87.102[CrossRef][GoogleScholar][Reflist]

[8]. Zhao X., Lin C. W., Wang J., Oh D. H. (2014). Advances in rapid detection methods for foodborne pathogens. J. Microbiol. Biotechn. 24, 297–312. 10.4014/jmb.1310.10013 [PubMed] [CrossRef] [Google Scholar

[9]. Zhang G. (2013). Foodborne pathogenic bacteria detection: an evaluation of current and developing methods. Meducator 1, 15. [Google Scholar]