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Bacteriological Analytical Manual Online

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Appendix 1 Rapid Methods for Detecting Foodborne Pathogens

Author

(Return to Table of Contents)

Introduction

Authors Note: This section differs from others in this manual in that it lists methods that are not necessarily used by FDA. In addition, the detailed protocols for these methods are not presented, and the user is referred to the instructions that accompany the test kits. One reason for this departure is the incremental rate of change and innovation in rapid testing technology. The best of these new techniques should be evaluated individually by user labs for their particular needs, and also collaboratively for possible adoption as official methods by the AOAC International (1).

The following text and tables list many of the commercially available rapid methods; they are classified by the principles underlying the procedure used. The assay principles and some of the detailed procedures are discussed in other chapters of this manual and/or in the literature cited in the tables. The AOAC status of rapid tests is indicated for those methods that have been validated or evaluated by AOAC (1) and have been adopted as AOAC Official methods. However, these methods continue to be modified or adapted, so that published information may not be the most current. Rapid methods are generally used as screening techniques, with negative results accepted as is, but positive results requiring **confirmation** by the appropriate official method, which, in many instances, is cultural. In many other instances, the rapid method has not been validated; therefore, the listing of a method or kit in this chapter in no way constitutes FDA recommendation or approval.

Rapid Methods

The rapid detection of pathogens and other microbial contaminants in food is critical for ensuring the safety of consumers. Traditional methods to detect foodborne bacteria often rely on time-consuming growth in culture media, followed by isolation, biochemical identification, and sometimes serology. Recent advances in technology make detection and identification faster, more convenient, more sensitive, and more specific than conventional assays -- at least in theory. These

new methods are often referred to as "rapid methods", a subjective term used loosely to describe a vast array of tests that includes miniaturized biochemical kits, antibody- and DNA-based tests, and assays that are modifications of conventional tests to speed up analysis (8, 15, 16, 24, 36). Some of these assays have also been automated to reduce hands-on manipulations. With few exceptions, almost all assays used to detect specific pathogens in foods require some growth in an enrichment medium before analysis.

Experts who were surveyed in 1981 (19) about future developments in methods used for food microbiology, accurately predicted the widespread use of miniaturized biochemical kits for the identification of pure cultures of bacteria isolated from food. Most consist of a disposable device containing 15 - 30 media or substrates specifically designed to identify a bacterial group or species. With the exception of a few kits where results can be read in 4 hrs, most require 18-24 hrs incubation. In general, miniaturized biochemical tests are very similar in format and performance, showing 90-99% accuracy in comparison to conventional methods (5, 16, 21). However, kits that have been in use longer may have a more extensive identification database than newer tests. Most miniaturized kits are designed for enteric bacteria, but kits for the identification of non-*Enterobacteriaceae* are also available, including for *Campylobacter*, *Listeria*, anaerobes, non-fermenting Gram-negative bacteria and for Gram-positive bacteria (Table 1).

Advances in instrumentation have enabled automation of the miniaturized biochemical identification tests. These instruments can incubate the reactions and automatically monitor biochemical changes to generate a phenotypic profile, which is then compared with the provided database stored in the computer to provide an identification (8, 23, 35). Other automated systems identify bacteria based on compositional or metabolic properties, such as fatty acid profiles, carbon oxidation profiles (28) or other traits (Table 1).

Not forecast in that 1981 survey were the potential applications of immunological and genetic techniques in food microbiology (19). During the 1980s, major advances in basic research were transferred rapidly to applied areas, as "biotechnology" companies emerged and sought markets in the diagnostic field (11). DNA and antibody-based assays for numerous microbes or their toxins are now available commercially (12).

There are many DNA-based assay formats, but only probes, PCR and bacteriophage have been developed commercially for detecting foodborne pathogens. Probe assays generally target ribosomal RNA (rRNA), taking advantage of the fact that the higher copy number of bacterial rRNA provides a naturally amplified target and affords greater assay sensitivity (6, 14, 25, 37) (Table 2).

The basic principle of DNA hybridization is also being utilized in other technologies, such as the polymerase chain reaction (PCR) assay, where short fragments of DNA (probes) or primers are hybridized to a specific sequence or template, which is then enzymatically amplified by *Taq* polymerase using a thermocycler (2, 22). Theoretically, PCR can amplify a single copy of DNA by a million fold in less than 2 hrs; hence its potential to eliminate, or greatly reduce the need for cultural enrichment. However, the presence of inhibitors in foods and in many culture media can prevent primer binding and diminish amplification efficiency (26, 34), so that the extreme sensitivity achievable by PCR with pure cultures is often reduced when testing foods. Therefore, some cultural enrichment is still required prior to analysis (Table 2).

The highly specific interaction of phage with its bacterial host has also been used to develop assays

for foodborne pathogens (<u>38</u>). One example is an assay for *Salmonella*, in which a specific bacteriophage was engineered to carry a detectable marker (ice nucleation gene). In the presence of *Salmonella*, the phage confers the marker to the host, which then expresses the phenotype to allow detection (<u>Table 2</u>).

The highly specific binding of antibody to antigen, especially monoclonal antibody, plus the simplicity and versatility of this reaction, has facilitated the design of a variety of antibody assays and formats, and they comprise the largest group of rapid methods being used in food testing (3, 10, 12, 33). There are 5 basic formats of antibody assays (12), the simplest of which is latex agglutination (LA), in which antibody-coated colored latex beads or colloidal gold particles are used for quick serological identification or typing of pure culture isolates of bacteria from foods (7, 12). A modification of LA, known as reverse passive latex agglutination (RPLA), tests for soluble antigens and is used mostly in testing for toxins in food extracts or for toxin production by pure cultures (12) (Table 3).

In the immunodiffusion test format, an enrichment sample is placed in a gel matrix with the antibody; if the specific antigen is present, a visible line of precipitation is formed (30).

The enzyme-linked immunosorbent assay (ELISA) is the most prevalent antibody assay format used for pathogen detection in foods $(\underline{3}, \underline{33})$. Usually designed as a "sandwich" assay, an antibody bound to a solid matrix is used to capture the antigen from enrichment cultures and a second antibody conjugated to an enzyme is used for detection. The walls of wells in microtiter plates are the most commonly used solid support; but ELISAs have also been designed using dipsticks, paddles, membranes, pipet tips or other solid matrices (12) (Table 3).

Antibodies coupled to magnetic particles or beads are also used in immunomagnetic separation (IMS) technology to capture pathogens from pre-enrichment media (<u>31</u>). IMS is analogous to selective enrichment, but instead of using antibiotics or harsh reagents that can cause stress-injury, an antibody is used to capture the antigen, which is a much milder alternative. Captured antigens can be plated or further tested using other assays.

Immunoprecipitation or immunochromatography, still another antibody assay format, is based on the technology developed for home pregnancy tests. It is also a "sandwich" procedure but, instead of enzyme conjugates, the detection antibody is coupled to colored latex beads or to colloidal gold. Using only a 0.1 ml aliquot, the enrichment sample is wicked across a series of chambers to obtain results (9). These assays are extremely simple, require no washing or manipulation and are completed within 10 minutes after cultural enrichment (Table 3).

The last mentioned "category" of rapid methods includes a large variety of assays, ranging from specialized media to simple modifications of conventional assays, which result in saving labor, time, and materials. Some, for instance, use disposable cardboards containing dehydrated media, which eliminates the need for agar plates, constituting savings in storage, incubation and disposal procedures (4, 5). Others incorporate specialized chromogenic and fluorogenic substrates in media to rapidly detect trait enzymatic activity (13, 17, 20, 27, 29). There are also tests that measure bacterial adenosine triphosphate (ATP), which (although not identifying specific species), can be used to rapidly enumerate the presence of total bacteria (Table 4).

Applications and Limitations of Rapid Methods

Almost all rapid methods are designed to detect a single target, which makes them ideal for use in quality control programs to quickly screen large numbers of food samples for the presence of a particular pathogen or toxin. A positive result by a rapid method however, is only regarded as presumptive and must be confirmed by standard methods (11). Although confirmation may extend analysis by several days, this may not be an imposing limitation, as negative results are most often encountered in food analysis.

Most rapid methods can be done in a few minutes to a few hours, so they are more rapid than traditional methods. But, in food analysis, rapid methods still lack sufficient sensitivity and specificity for direct testing; hence, foods still need to be culture-enriched before analysis (12). Although enrichment is a limitation in terms of assay speed, it provides essential benefits, such as diluting the effects of inhibitors, allowing the differentiation of viable from non-viable cells and allowing for repair of cell stress or injury that may have resulted during food processing.

Evaluations of rapid methods show that some perform better in some foods than others. This can be attributed mostly to interference by food components, some of which can be especially troublesome for the technologies used in rapid methods. For example, an ingredient can inhibit DNA hybridization or *Taq* polymerase, but has no effect on antigen-antibody interactions and the converse situation may also occur (12). Since method efficiencies may be food dependent, it is advisable to perform comparative studies to ensure that a particular assay will be effective in the analysis of that food type.

The specificity of DNA based assays is dictated by short probes; hence, a positive result, for instance with a probe or primers specific for a toxin gene, only indicates that bacteria with those gene sequences are present and that they have the potential to be toxigenic. But, it does not indicate that the gene is actually expressed and that the toxin is made. Likewise, in clostridial and staphylococcal intoxication, DNA probes and PCR can detect only the presence of cells, but are of limited use in detecting the presence of preformed toxins (12).

Currently, there are at least 30 assays each for testing for *E. coli* O157:H7 and for *Salmonella*. Such a large number of options can be confusing and overwhelming to the user, but, more importantly, has limited the effective evaluation of these methods. As a result, only few methods have been officially validated for use in food testing (1,11).

Conclusions

As a rapid method is used more frequently, its benefits and at the same time, its limitations also become more apparent. This section only briefly described some of the rapid method formats and selected problems encountered when using these assays in food analysis. However, because of the complex designs and formats of these tests, coupled with the difficulties of testing foods, users must exercise caution when selecting rapid methods and to also evaluate these tests thoroughly, as some may be more suitable than others for distinct testing situations or for assaying certain types of food. Lastly, technology continues to advance at a great pace and next generation assays, such as biosensors (18) and DNA chips (32) already are being developed that potentially have the capability for near real-time and on-line monitoring of multiple pathogens in foods.

NOTE: The listings provided in Tables 1-4 are intended for

general reference only and do not indicate endorsement or approval by FDA for use in food analysis.

Table 1. Partial list of miniaturized biochemical kits and automated systems for identifying foodborne bacteria* (5, 8, 15, 16, 21, 35, 36).

System	Format	Manufacturer	Organisms	
API ^b	biochemical	bioMerieux	<i>Enterobacteriaceae, Listeria,</i> <i>Staphylococcus, Campylobacter,</i> Non- fermenters, anaerobes	
Cobas IDA	biochemical	Hoffmann LaRoche	Enterobacteriaceae	
Micro-ID ^b	biochemical	REMEL	Enterobacteriaceae, Listeria	
EnterotubeII	biochemical	Roche	Enterobacteriaceae	
Spectrum 10	biochemical	Austin Biological	Enterobacteriaceae	
RapID	biochemical	Innovative Diag.	Enterobacteriaceae	
BBL Crystal	biochemical	Becton Dickinson	<i>Enterobacteriaceae, Vibrionaceae</i> , Non- fermenters, anaerobes	
Minitek	biochemical	Becton Dickinson	Enterobacteriaceae	
Microbact	biochemical	Microgen	<i>Enterobacteriaceae</i> , Gram negatives, Non-fermenters, <i>Listeria</i>	
Vitek ^b	biochemical ^a	bioMerieux	<i>Enterobacteriaceae</i> , Gram negatives, Gram positives	
Microlog	C oxidation ^a	Biolog	<i>Enterobacteriaceae</i> , Gram negatives, Gram positives	
MIS ^b	Fatty acid ^a	Microbial-ID	Enterobacteriaceae, Listeria, Bacillus, Staphylococcus, Campylobacter	
Walk/Away	biochemical ^a	MicroScan	Enterobacteriaceae, Listeria, Bacillus, Staphylococcus, Campylobacter	
Replianalyzer	biochemical ^a	Oxoid	Enterobacteriaceae, Listeria, Bacillus, Staphylococcus, Campylobacter	
Riboprinter	nucleic acid ^a	Qualicon	Salmonella, Staphylococcus, Listeria, Escherichia coli	
Cobas Micro- ID	biochemical ^a	Becton Dickinson	n <i>Enterobacteriaceae</i> , Gram negatives, Non- fermenters	
Malthus ^b	conductance ^a	Malthus	Salmonella, Listeria, Campylobacter, E. coli Pseudomonas, coliforms	
Bactometer	impedance ^a	bioMerieux	Salmonella	

* Table modified from: Feng, P., App.I., FDA Bacteriological Analytical Manual, 8A ed. ^a Automated systems

^b Selected systems adopted AOAC Official First or Final Action.

NOTE: This table is intended for general reference only and lists known available methods. Presence on this list does not indicate verification, endorsement, or approval by FDA for use in food analysis.

Table 2. Partial list of commercially-available, nucleic acid-based assays used in the detection of foodborne bacterial pathogens* (2, 5, 8, 12, 14, 22, 25, 36, 37).

Organism	Trade Name	Format	Manufacturer
Clostridium botulinum	Probelia	PCR	BioControl
Campylobacter	AccuProbe	probe	GEN-PROBE
	GENE-TRAK	probe	Neogen
Escherichia coli	GENE-TRAK	probe	Neogen
<i>E. coli</i> O157:H7	BAX	PCR ^a	Qualicon
	Probelia	PCR	BioControl
Listeria	GENE-TRAK ^c	probe	Neogen
	AccuProbe	probe	GEN-PROBE
	BAX	PCR	Qualicon
	Probelia	PCR	BioControl
Salmonella	GENE-TRAK ^c	probe	Neogen
	BAX	PCR	Qualicon
	BIND ^b	phage	BioControl
	Probelia	PCR	BioControl
Staphylococcus aureus	AccuProbe	probe	GEN-PROBE
	GENE-TRAK	probe	Neogen
Yersinia enterocolitica	GENE-TRAK	probe	Neogen

* Table modified from: Feng, P., App.I, FDA Bacteriological Analytical Manual, 8A ed.

^a Polymerase chain reaction

^b Bacterial Ice Nucleation Diagnostics

^c Adopted AOAC Official First or Final Action

NOTE: This table is intended for general reference only and lists known available methods. Presence on this list does not indicate verification, endorsement, or approval by FDA for use in food analysis.

Table 3. Partial list of commercially-available, antibody-based assays for the detection of foodborne pathogens and toxins* (3, 5, 8, 12, 33, 36).

Organism/toxin	Trade Name	Assay Format ^a	Manufacturer
<i>Bacillus cereus</i> diarrhoeal toxin	TECRA	ELISA	TECRA
	BCET	RPLA	Unipath
Campylobacter	Campyslide	LA	Becton Dickinson
	Meritec-campy	LA	Meridian
	MicroScreen	LA	Mercia
	VIDAS	ELFA ^b	bioMerieux
	EiaFOSS	ELISA ^b	Foss
	TECRA	ELISA	TECRA
<i>Clostridium botulinum</i> toxin	ELCA	ELISA	Elcatech
C. perfringens enterotoxin	PET	RPLA	Unipath
Escherichia coli			
EHEC ^{**} ^c O157:H7	RIM	LA	REMEL
	E. coli O157	LA	Unipath
	Prolex	LA	PRO-LAB
	Ecolex O157	LA	Orion Diagnostica
	Wellcolex O157	LA	Murex
	E. coli O157	LA	TechLab
	O157&H7	sera	Difco
	PetrifilmHEC	Ab-blot	3M
	EZ COLI	Tube-EIA	Difco
	Dynabeads	Ab-beads	Dynal
	EHEC-TEK	ELISA	Organon-Teknika
	Assurance ^e	ELISA	BioControl
	HECO157	ELISA	3M Canada
	TECRA	ELISA	TECRA
	E. coli O157	ELISA	LMD Lab
	Premier O157	ELISA	Meridian
	<i>E. coli</i> O157:H7	ELISA	Binax
	E. coli Rapitest	ELISA	Microgen
	Transia Card E. coli O157	ELISA	Diffchamb
	E. coli O157	EIA/capture	TECRA

	VIP ^e	Ab-ppt	BioControl
	Reveal	Ab-ppt	Neogen
	Quix Rapid O157	Ab-ppt	Universal HealthWatch
	ImmunoCardSTAT	Ab-ppt	Meridian
	VIDAS	ELFA ^b	bioMerieux
	EiaFOSS	ELISA ^b	Foss
Shiga toxin (Stx)	VEROTEST	ELISA	MicroCarb
	Premier EHEC	ELISA	Meridian
	Verotox-F	RPLA	Denka Seiken
ETEC ^c			
Labile toxin (LT)	VET-RPLA	RPLA	Oxoid
Stabile toxin (ST)	E. coli ST	ELISA	Oxoid
Listeria	Microscreen	LA	Microgen
	Listeria Latex	LA	Microgen
	Listeria-TEK ^e	ELISA	Organon Teknika
	TECRA ^e	ELISA	TECRA
	Assurance ^e	ELISA	BioControl
	Transia Plate Listeria	ELISA	Diffchamb
	Pathalert	ELISA	Merck
	Listertest	Ab-beads	VICAM
	Dynabeads	Ab-beads	Dynal
	VIP ^e	Ab-ppt	BioControl
	Clearview	Ab-ppt	Unipath
	RAPIDTEST	Ab-ppt	Unipath
	VIDAS ^e	ELFA ^b	bioMerieux
	EiaFOSS	ELISA ^b	Foss
	UNIQUE	Capture-EIA	TECRA
Salmonella	Bactigen	LA	Wampole Labs
	Spectate	LA	Rhone-Poulenc
	Microscreen	LA	Mercia
	Wellcolex	LA	Laboratoire Wellcome

	Serobact	LA	REMEL
	RAPIDTEST	LA	Unipath
	Dynabeads	Ab-beads	Dynal
	Screen	Ab-beads	VICAM
	CHECKPOINT	Ab-blot	KPL
	1-2 Test ^e	diffusion	BioControl
	SalmonellaTEK ^e	ELISA	Organon Teknika
	TECRA ^e	ELISA	TECRA
	EQUATE	ELISA	Binax
	BacTrace	ELISA	KPL
	LOCATE	ELISA	Rhone-Poulenc
	Assurance ^e	ELISA	BioControl
	Salmonella	ELISA	GEM Biomedical
	Transia Plate Salmonella Gold	ELISA	Diffchamb
	Bioline	ELISA	Bioline
	VIDAS ^e	ELFA ^b	bioMerieux
	OPUS	ELISA ^b	TECRA
	PATH-STIK	Ab-ppt	LUMAC
	Reveal	Ab-ppt	Neogen
	Clearview	Ab-ppt	Unipath
	UNIQUE ^e	Capture-EIA	TECRA
Shigella	Bactigen	LA	Wampole Labs
	Wellcolex		Laboratoire Wellcome
Staphylococcus aureus	Staphyloslide	LA	Becton Dickinson
	AureusTest ^e	LA	Trisum
	Staph Latex	LA	Difco
	S. aureus VIA	ELISA	TECRA
enterotoxin	SET-EIA	ELISA	Toxin Technology
	SET-RPLA	RPLA	Unipath
	TECRA ^e	ELISA	TECRA
	Transia Plate SE	ELISA	Diffchamb

	RIDASCREEN	ELISA	R-Biopharm
	VIDAS	ELFA ^b	bioMerieux
	OPUS	ELISA ^b	TECRA
Vibrio cholera	choleraSMART	Ab-ppt	New Horizon
	bengalSMART	Ab-ppt	New Horizon
	choleraScreen	Agglutination	New Horizon
	bengalScreen	Agglutination	New Horizon
enterotoxin	VET-RPLA ^d	RPLA	Unipath

* Table modified from: Feng, P., App.I, FDA Bacteriological Analytical Manual, 8A ed.

^a Abbreviations: ELISA, enzyme linked immunosorbent assay; ELFA, enzyme linked fluorescent assay; RPLA, reverse passive latex agglutination; LA, latex agglutination; Ab-ppt, immunoprecipitation.

^b Automated ELISA

^c EHEC - Enterohemorrhagic *E. coli*; ETEC - enterotoxigenic *E. coli*

^d Also detects *E. coli* LT enterotoxin

^e Adopted AOAC Official First or Final Action

****** CAUTION: unless the assays claim that they are specific for the O157:H7 serotype, most of these tests detect only the O157 antigen; hence will also react with O157 strains that are not of H7 serotype. These O157, non-H7 strains, generally do not produce Shiga toxins and are regarded as not pathogenic for humans. Furthermore, some antibodies to O157 can also cross react with *Citrobacter*, *E. hermanii* and other enteric organisms.

NOTE: This table is intended for general reference only and lists known available methods. Presence on this list does not indicate verification, endorsement, or approval by FDA for use in food analysis.

Organism	Trade Name	Format ^a	Assay Manufacturer
Bacteria	Redigel ^b	Media	RCR Scientific
	Isogrid ^b	HGMF	QA Labs
	Enliten	ATP	Promega
	Profile-1	ATP	New Horizon
	Biotrace	ATP	Biotrace
	Lightning	ATP	Idexx
	Petrifilm ^b	media-film	3M

Table 4. Partial list of other commercially available rapid methods and specialty substrate media for detection of foodborne bacteria* (4, 8, 13, 20, 27, 36).

	Sim Plate	media	Idexx
Coliform/ E. coli	Isogrid ^b	HGMF/MUG	QA Labs
	Petrifilm ^b	media-film	3M
	SimPlate	media	Idexx
	Redigel	Media	RCR Scientific
	ColiQuik ^c	MUG/ONPG	Hach
	ColiBlue ^c	media	Hach
	Colilert ^{b,c}	MUG/ONPG	Idexx
	LST-MUG ^b	MPN media	Difco & GIBCO
	ColiComplete ^b	MUG-Xgal	BioControl
	Colitrak	MPN-MUG	BioControl
	ColiGel & E*Colite ^c	MUG-Xgal	Charm Sciences
	CHROMagar	Medium	CHROMagar
E. coli	MUG disc	MUG	REMEL
	CHROMagar	Medium	CHROMagar
EHEC ^d	Rainbow Agar	Medium	Biolog
	BCMO157:H7	Medium	Biosynth
	Fluorocult O157:H7	Medium	Merck
Listeria monocytogenes	BCM	Medium	Biosynth
	Isogrid ^b	HGMF	QA Labs
	OSRT	Medium/ motility	Unipath (Oxoid)
C 1 11	Rambach	Medium	CHROMagar
Salmonella	MUCAP	C8esterase	Biolife
	XLT-4	Medium	Difco
	MSRV ^b	Medium	
Yersinia	Crystal violet	Dye binding	Polysciences

* Table modified from: Feng, P., App.I, FDA Bacteriological Analytical Manual, 8A ed.

^a Abbreviations: APC, aerobic plate count; HGMF, hydrophobic grid membrane filter; ATP, adenosine triphosphate; MUG, 4-methylumbelliferyl-β-D-glucuronide; ONPG, O-nitrophenyl β-D-galactoside; MPN, most probable number.

^b Adopted AOAC Official First or Final Action.

^c Application for water analysis

^d EHEC - enterohemorrhagic *Escherichia coli*

NOTE: This table is intended for general reference only and lists known available methods.

Presence on this list does not indicate verification, endorsement, or approval by FDA for use in food analysis.

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