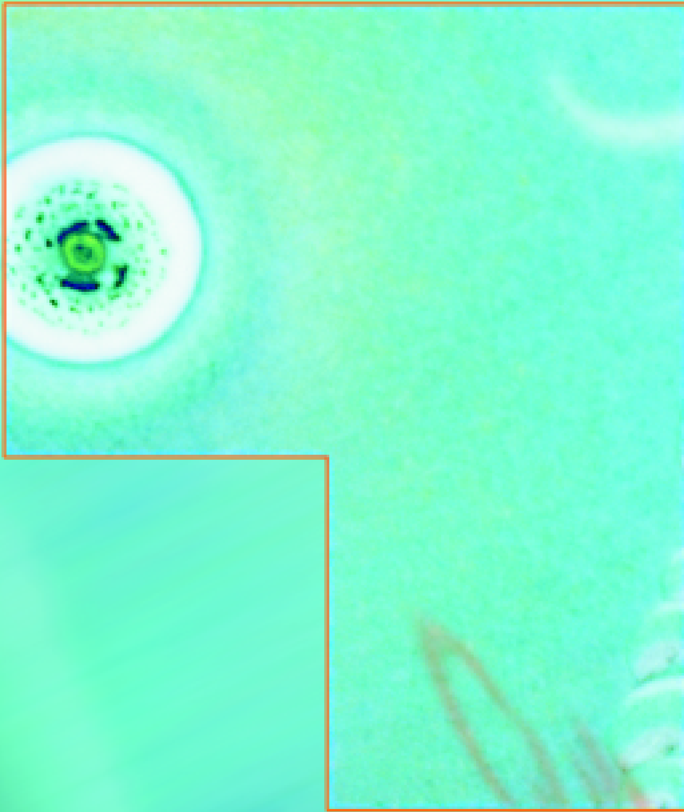
3



Culture Media, Prepared

Bacto® ATS Medium

Intended Use

Bacto ATS Medium is used for the isolation and cultivation of mycobacteria.

Also Known As

ATS Medium is also known as American Trudeau Society Medium.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980's the number of tuberculosis (TB) cases in the U.S. began increasing. Before this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984. Non-tuberculous mycobacteria infections have also increased since the mid 1980's.[2]

Two types of semi-solid culture media are available for the isolation of mycobacteria: egg-based media and agar-based media. Most formulations for the isolation of mycobacteria include malachite green, which is used to inhibit contaminating organisms.

Principles of the Procedure

ATS Medium is prepared according to the formula described by the committee on evaluation of Laboratory Procedures of the American Trudeau Society.[3] ATS Medium is an egg-based medium containing a small amount of Malachite Green. Due to the low concentration of Malachite Green, this formulation permits the relatively early detection of mycobacteria colonies. This medium is well suited to specimens that are not heavily contaminated such as cerebral spinal fluid (CSF) and pleural fluid.[4,5] When present, contaminating microorganisms may liquefy the medium.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Not applicable
Prepared Medium:	Light green, opaque
Reaction of Medium at 25°C:	pH 6.4 -7.5

Cultural Response

Inoculate and incubate at 35 ± 2°C under CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium fortuitum	6841	100-1,000	good
Mycobacterium intracellulare	13950	100-1,000	good
Mycobacterium kansasii	12478	100-1,000	good
Mycobacterium tuberculosis H37Ra	25177	100-1,000	good
Mycobacterium scrofulaceum	19981	100-1,000	good

These cultures are the minimum that should be used for performance testing.

Formula

ATS Medium

Formula Per Liter

Egg Yolk Suspension	500 ml
Glycerol Extract of Potatoes	500 ml
Malachite Green	0.2 ml

Final pH 6.4-7.5 at 25°C

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

ATS Medium

Materials Required But Not Provided

Incubator

Specimen Collection and Preparation[5]

1. Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate the specimen into medium.

Test Procedure

1. Incubate tubes for up to eight weeks.
2. Examine tubes for growth.

Results

Observe for colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Limitations of the Procedure

Negative culture results do not rule out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures include the following:

1. The specimen was not representative of the infectious material, i.e. saliva instead of sputum.
2. The mycobacteria were destroyed during digestion and decontamination of the specimen.
3. Gross contamination interfered with the growth of the mycobacteria.
4. Proper aerobic CO_2 tension was not provided during incubation.

References

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Packaging

ATS Medium	100 tubes	1019-79*

Bacto® Dubos Albumin Broth

See page 163 of Section II for complete listing of this product.

HYcheck™

HYcheck D/E Neutralizing Agar · HYcheck for Disinfection Control · HYcheck for Enterobacteriaceae · HYcheck Plate Count Agar With TTC · HYcheck for Total Count · HYcheck for Yeasts and Molds · HYcheck for Yeasts and Molds with TTC

Intended Use

HYcheck is a hygiene contact slide which is used for assessing the microbiological contamination of surfaces or fluids.

Summary and Explanation

Monitoring the microbial flora of environmental surfaces, walls, ceilings, and equipment is an important stage in achieving good manufacturing practices in factories handling foods, cosmetics or pharmaceuticals.[1,2,3] To maintain good hygiene standards in hotels and restaurant kitchens, microbiological contamination must also be monitored.[4] Methods to monitor the environmental flora have been described using either swabbing techniques[5] or contact plates.[6] Contact slides were created to monitor the microbial flora of liquids (e.g. urine, milk) and equipment surfaces in the clinical and food industries.[1] Contact slides are statistically comparable to swab and contact plates for surface sampling.[1] The HYcheck contact slides were developed for the testing of fluids and surfaces for microbial cleanliness.

HYcheck is a double sided, hinged plastic paddle containing two agar surfaces. The agar surface extends above the paddle allowing for contact with test surfaces. The hinged paddle allows the agar surface to be easily held against each test area during sampling. The surface area of the paddle is clearly divided into seven units of one centimeter each to allow direct counting of microbial density per unit area.

The HYcheck range of hygiene control slides consists of seven media combinations designed to meet the various needs for monitoring different types of microbial contamination.

HYcheck D/E Neutralizing Agar has both sides coated with D/E Neutralizing Agar, a medium developed by Dey and Engley[7] to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals. D/E Neutralizing Agar neutralizes higher concentrations of residual antimicrobials, when compared with other standard neutralizing formulas, such as Letheen media, Thioglycollate media, and Neutralizing Buffer.[8,9] Complete neutralization of disinfectants is important because disinfectant carryover can result in a false no growth result. D/E Neutralizing media effectively neutralize the inhibitory effects of disinfectant carryover,[10,11] allowing differentiation between bacteriostasis and true bactericidal actions of disinfectant chemicals.

User Quality Control

Identity Specifications

HYcheck D/E Neutralizing Agar

Medium:	**D/E Neutralizing Agar**
Appearance:	Lavender
Microbial Limits Test:	Satisfactory
pH at 25°C:	7.6 ± 0.2

HYcheck for Disinfection Control

Media:	**D/E Neutralizing Agar**	**Tryptic Soy Agar**
Appearance:	Lavender	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.6 ± 0.2	7.3 ± 0.2

continued on following page

HYcheck for Enterobacteriaceae

Media:	**Violet Red Bile Glucose Agar**	**Tryptic Soy Agar**
Appearance:	Reddish purple	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.4 ± 0.2	7.3 ± 0.2

HYcheck Plate Count Agar with TTC

Medium:	**Plate Count Agar with 0.01% TTC**
Appearance:	Light amber
Microbial Limits Test:	Satisfactory
pH at 25°C:	7.0 ± 0.2

HYcheck for Total Count

Media:	**Plate Count Agar**	**Plate Count Agar with 0.01% TTC**
Appearance:	Light amber	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.0 ± 0.2	7.0 ± 0.2

HYcheck for Yeasts and Molds

Media:	**Rose Bengal Chloramphenicol Agar**	**Tryptic Soy Agar**
Appearance:	Rose pink	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.2 ± 0.2	7.3 ± 0.2

HYcheck for Yeast and Molds with TTC

Media:	**Rose Bengal Chloramphenicol Agar**	**Tryptic Soy Agar with 0.01% TTC**
Appearance:	Rose pink	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.2 ± 0.2	7.3 ± 0.2

Cultural Response (approx inoculum 30-300 CFU)

HYcheck D/E Neutralizing Agar

Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON D/E AGAR
Aspergillus niger NCPF 2275	–	good
Bacillus subtilis	6633	good
Candida albicans	2091	good
Escherichia coli	25922*	good
Pseudomonas aeruginosa	27853*	good
Staphylococcus aureus	25923*	good
Staphylococcus epidermidis	12228*	good

HYcheck for Disinfection Control

Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON D/E	GROWTH ON TSA
Aspergillus niger NCPF 2275	–	good	good
Bacillus subtilis	6633	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	good	good
Pseudomonas /nosa	27853*	good	good
Staphylococcus aureus	25923*	good	good
Staphylococcus epidermidis	12228*	good	good

HYcheck for Enterobacteriaceae

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON VRBGA	GROWTH ON TSA
Enterobacter aerogenes	13048*	good	good
Enterococcus faecalis	19433*	none to poor	good
Escherichia coli	25922*	good	good
Proteus mirabilis NCTC 11938	–	good	good
Salmonella typhimurium	14028*	good	good
Shigella sonnei	25931*	good	good
Staphylococcus aureus	25923*	none to poor	good

HYcheck Plate Count Agar with TTC

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON PCA W/TTC
Enterococcus faecalis	19433*	good
Escherichia coli	25922*	good
Proteus vulgaris	13315	good
Salmonella typhimurium	14028*	good
Staphylococcus aureus	25923*	poor

HYcheck for Total Count

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON PCA	GROWTH ON PCA W/TTC
Enterococcus faecalis	19433*	good	good
Escherichia coli	25922*	good	good
Proteus vulgaris	13315	good	good
Salmonella typhimurium	14028*	good	good
Staphylococcus aureus	25923*	good	poor

HYcheck for Yeasts and Molds

Inoculate and incubate at 30 ± 2ºC for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON RBCA	GROWTH ON TSA
Aspergillus niger NCPF 2275	–	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	none to poor	good
Saccharomyces cerevisiae NCYC 1211	–	good	good
Serratia marcescens	8100	none to poor	good
Staphylococcus aureus	25923*	none to poor	good
Streptococcus pyogenes	19615*	none to poor	good

HYcheck for Yeasts and Molds with TTC

Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON RBCA	GROWTH ON TSA W/TTC
Aspergillus niger NCPF 2275	—	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	none to poor	good
Saccharomyces cerevisiae NCYC 1211	–	good	poor
Serratia marcescens	8100	none to poor	good
Staphylococcus aureus	25923*	none to poor	poor
Streptococcus pyogenes	19615*	none to poor	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

HYcheck for Disinfection Control has side one coated with D/E Neutralizing Agar (D/E) (see above), and side two coated with Tryptic Soy Agar (TSA). In 1955, Leavitt et al.[12] demonstrated that Tryptic Soy Agar supports excellent growth of a both aerobic and anaerobic microorganisms. Tryptic Soy Agar is a general purpose medium that is recommended in multiple water and wastewater applications.[13]

HYcheck for Enterobacteriaceae has side one coated with Violet Red Bile Glucose Agar and side two coated with Tryptic Soy Agar, a general purpose growth medium. Violet Red Bile Glucose Agar is a selective medium used for the enumeration of *Enterobacteriaceae* in foods. Coliform bacteria have long been used as an index of fecal contamination in waters, and their presence in milk is used as an index of sanitation in milk processing.[14] The presence of *Enterobacteriaceae*, coliforms, *Salmonellae*, *Klebsiella* or *Citrobacter*, in raw foodstuffs is an indicator of fecal contamination. Their presence after processing may indicate a failure in the manufacturing process.

HYcheck Plate Count Agar with TTC has both sides coated with Plate Count Agar with TTC (0.01% 2,3,5-Triphenyl Tetrazolium Chloride).

HYcheck for Total Count has side one coated with Plate Count Agar and side two coated with Plate Count Agar with 0.01% TTC. Plate Count Agar is used for enumerating bacteria in water, wastewater, food and dairy products.[13,15-18] TTC is a redox indicator that is colorless in the oxidized form. TTC is reduced to insoluble triphenylformazan by certain actively metabolizing bacteria, resulting in a red color in the presence of bacterial growth.

There are two HYcheck products for yeasts and molds: 1) **HYcheck for Yeasts and Molds** has side one coated with Rose Bengal Chloramphenical Agar and side two coated with Tryptic Soy Agar; 2) **HYcheck for Yeasts and Molds with TTC** has side one coated with Rose Bengal Chloramphenical Agar and side two coated with Tryptic Soy Agar with 0.01% TTC. Rose Bengal Chloramphenical Agar is recommended in the selective isolation and enumeration of yeasts and molds from environmental materials and foodstuffs. The pH of the medium is near neutrality for improved growth and recovery of acid sensitive strains. [19-21]

Principles of the Procedure

HYcheck D/E Neutralizing Agar

Tryptone provides carbon and nitrogen. Yeast Extract provides vitamins, cofactors and additional nitrogen and carbon. Dextrose provides fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Brom Cresol Purple is a colorimetric indicator. Bacto Agar is a solidifying agent.

HYcheck for Disinfection Control

D/E Neutralizing Agar (D/E) - side one

Tryptone provides carbon and nitrogen. Yeast Extract provides vitamins, cofactors and additional nitrogen and carbon. Dextrose provides fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Brom Cresol Purple is a colorimetric indicator. Bacto Agar is a solidifying agent.

Tryptic Soy Agar (TSA) - side two

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

HYcheck for Enterobacteriaceae

Violet Red Bile Glucose Agar (VRBGA) - side one

Yeast Extract provides vitamins, cofactors, nitrogen and carbon. Glucose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

HYcheck Plate Count Agar (PCA) with TTC

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

HYcheck for Total Count

Plate Count Agar - side one

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

Plate Count Agar with TTC - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

HYcheck for Yeasts and Molds

Rose Bengal Chloramphenicol Agar (RBCA) - side one

Soytone provides carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Rose Bengal and Chloramphenicol inhibit bacterial growth and restrict size and height of rapidly growing mold colonies. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

HYcheck for Yeasts and Molds with TTC

Rose Bengal Chloramphenicol Agar - side one

Soytone provides carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Rose Bengal suppresses bacterial growth and restricts size and height of rapidly growing mold colonies. Chloramphenicol inhibits bacteria. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

Precautions

1. Do not touch agar surface.
2. Do not use if there are signs of dehydration or contamination.

Storage

Store HYcheck slides at 2-15°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

(One type is provided per package.)

HYcheck D/E Neutralizing Agar
HYcheck for Disinfection Control
HYcheck for Enterobacteriaceae
HYcheck Plate Count Agar with TTC
HYcheck for Total Count
HYcheck for Yeasts and Molds
HYcheck for Yeasts and Molds with TTC.

Test Procedure

Surfaces

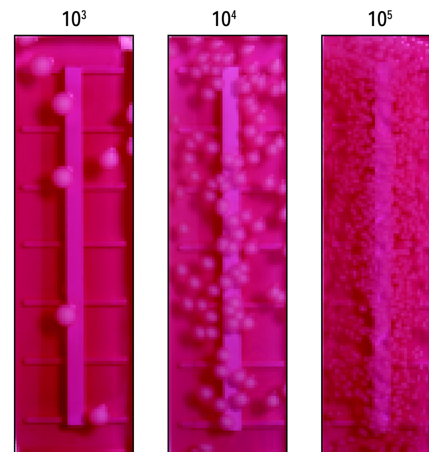
1. Loosen cap and remove HYcheck slide from the container.
2. Examine for dehydration or contamination.
3. Hold terminal spike against surface to be tested.
4. Press down on the spike to bend the paddle around the hinge line.
5. Gently lower the slide and press agar into contact with the test surface.
6. Apply firm and even pressure on the test surface for a few seconds.
7. Repeat procedure using the second agar surface on an area adjacent to the initial test site.
8. Replace slide in the container and close tightly.
9. Incubate in an upright position at indicated temperature.

Liquids

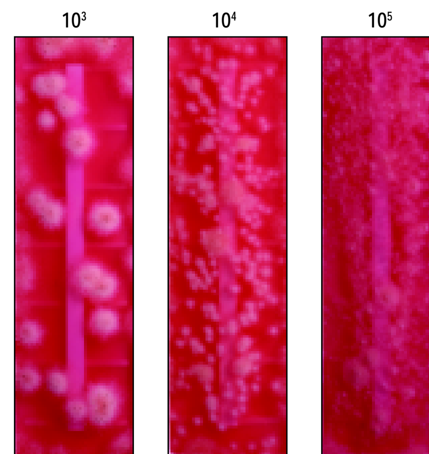
1. Loosen cap and remove HYcheck Slide from the container.
2. Examine for dehydration or contamination.
3. Immerse slide into test fluid so that agar surface becomes totally covered (if insufficient liquid is available, pour over surface of the slide).
4. Allow to drain.
5. Replace slide in the container and close tightly.
6. Incubate in an upright position at indicated temperature.

Results

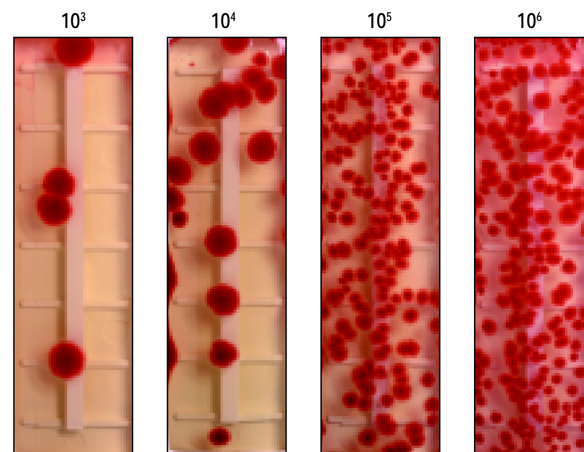
The following photos are reactions to *Candida albicans, Aspergillus niger and Escherichia coli.*



Candida albicans ATCC® 60193
on Rose Bengal Chloramphenicol Agar



Aspergillus niger ATCC® 1015
on Rose Bengal Chloramphenicol Agar



Escherichia coli ATCC® 11229
on Tryptic Soy Agar with 0.01% TTC

Limitations of the Procedure

1. Do not use the HYcheck Slide if it is contaminated or the agar medium is significantly dehydrated.

References

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Packaging

HYcheck D/E Neutralizing Agar	20 units	9041-36
HYcheck for Disinfection Control	20 units	9039-36
HYcheck for Enterobacteriaceae	20 units	9037-36
HYcheck Plate Count Agar with TTC	20 units	9045-36
HYcheck for Total Count	20 units	9053-36
HYcheck for Yeasts and Molds	20 units	9038-36
HYcheck for Yeasts and Molds with TTC	20 units	9046-36

Bacto® Petragnani Medium

Intended Use

Bacto Petragnani Medium is used for isolating and cultivating mycobacteria.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980s. the number of tuberculosis (TB) cases in the U.S. began increasing. Before this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984.[2] Non-tuberculous mycobacterial infections have also increased since the mid 1980s.[3]

Two types of semi-solid culture media are available for the isolation of mycobacteria, egg-based media and agar-based media. Most formulations for the isolation of mycobacteria include malachite green, which is used to inhibit contaminating organisms.

Petragnani Medium is an egg-based medium that is a modification of Petragnani[4] medium described by Norton, Thomor and Broom.[5] The formulation contains a large amount of malachite green which

inhibits the growth of contaminating organisms. This medium is well suited to specimens that are from nonsterile areas that may be heavily contaminated.[3, 6]

Principles of the Procedure

Whole Milk, Whole Eggs and Egg Yolks are protein sources. Potatoes and Potato Flour are starches that provide a carbohydrate source. Glycerol is a carbon source. Malachite Green inhibits contaminating organisms.

Formula

Petragnani Medium

Formula Per Liter

Whole Milk	900	ml
Potato Flour	36	g
Potato	500	g
Whole Eggs	1200	ml
Egg Yolks	115	ml
Bacto Glycerol	70	ml
Bacto Malachite Green	1.2	g

pH 7.2 ± 0.2 at 25°C

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store prepared medium at 2-8°C

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Prepared Appearance: Light to medium green, opaque, smooth slants with no visible contamination.

Reaction of Medium at 25°C: pH 7.2 ± 0.2

Cultural Response

Inoculate and incubate at 35 ± 2°C under CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	partial to complete inhibition
Mycobacterium fortuitum	6841	100-1,000	good
Mycobacterium intracellulare	13950	100-1,000	good
Mycobacterium kansasii	12478	100-1,000	good
Mycobacterium tuberculosis H37Ra	25177	100-1,000	good

These cultures are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Procedure

Materials Provided

Petragnani Medium

Materials Required But Not Provided

Specimen decontaminant and digestant
Buffer
Bovine albumin
Centrifuge
Inoculating Needles
CO_2 Incubator (35°C)

Method of Preparation

Supplied ready to use

Specimen Collection and Preparation[4]

1. Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate the specimen onto the medium.

Test Procedure

1. Incubate tubes for up to eight weeks.
2. Examine tubes for growth.

Results

Observe for colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Limitations of the Procedure

Negative culture results do not rule out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:

- The specimen was not representative of the infectious material, i.e., saliva instead of sputum.
- The mycobacteria were destroyed during digestion and decontamination of the specimen.
- Gross contamination interfered with the growth of the mycobacteria.
- Proper aerobic CO_2 tension was not provided during incubation.

References

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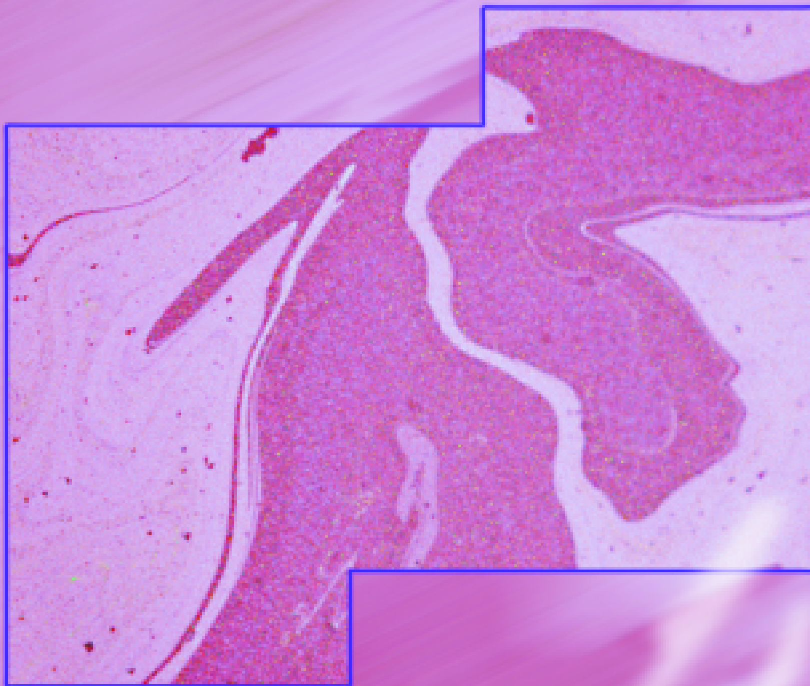
Packaging

Petragnani Medium	100 tubes	1010-79

Stains and Indicators



4

Bacto® Acridine Orange Stain
SpotTest™ Acridine Orange Stain

Intended Use

Bacto Acridine Orange Stain and SpotTest™ Acridine Orange Stain are used for detecting microorganisms in direct smears by the fluorescent staining technique.

Summary and Explanation

Fluorochromatic staining of microorganisms using acridine orange was first described by Strugger and Hilbrich in 1942[1] and has been used in the microscopic examination of soil and water.[2,3] Acridine orange possesses differential staining properties with regard to clinical materials when prepared at a low pH.[4] Bacteria stain bright orange and are differentiated from human cells and tissue debris which stain pale green to yellow.

Acridine orange staining is a simple, rapid, inexpensive alternative to blind subcultures.[5] The stain is more sensitive than the Gram stain for detecting microorganisms in clinical materials at concentrations of approximately 1 x 10^4 colony-forming units per ml.[6]

Acridine orange at a low pH has been used for the detection of *Trichomonas vaginalis*[7] and *Neisseria gonorrhoeae*[8] in clinical materials and for the enumeration of mycoplasmas.[9] The stain may be useful in the rapid screening of normally sterile specimens, such as cerebrospinal fluid where few organisms may be present, and in the rapid examination of blood smears or smears containing proteinaceous material, where differentiation of organisms from background material may be difficult.[10]

User Quality Control

Identity Specifications

Acridine Orange Stain
SpotTest™ Acridine Orange Stain

Solution: The solution should be clear, orange, and without evidence of a precipitate.

Reaction at 25°C: pH 3.5-4.0

Cultural Response

Prepare slides of the test organisms and sheep blood stained using Acridine Orange Stain or SpotTest™ Acridine Orange Stain. Examine slides using a fluorescent microscope at 1000X magnification.

ORGANISM	ATCC®	STAINED BACTERIA
Escherichia coli	25922*	orange to red-orange rods
Enterococcus faecalis	33186	orange to red-orange cocci

Background for both organisms: staining is hazy black or green; red blood cell "ghosts" stain pale green or have a green periphery.

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Acridine orange is a fluorochromatic dye that binds to the nucleic acids of bacteria and other cells.[11] Under UV light, Acridine Orange stains RNA and single-stranded DNA orange; double-stranded DNA appears green.

Formula

Acridine Orange Stain
SpotTest™ Acridine Orange Stain

Formula Per Liter

Acridine Orange	0.1 g
Acetate Buffer, 0.5M	1 liter

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store at 15-30°C. Acridine Orange Stain is light sensitive. Protect from light.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Acridine Orange Stain
SpotTest™ Acridine Orange Stain
Ampule Crusher

Materials Required But Not Provided

Glass microscope slides
Methanol
Fluorescent microscope suitable for use with Acridine Orange

Method of Preparation

Not applicable

Specimen Collection and Preparation

Not applicable

Preparation, Staining, and Examination of Smears

1. Prepare a smear of the specimen to be stained on a clean glass slide.
2. Allow to air dry.
3. Fix smear with 50% or 100% methanol for 1 to 2 minutes.
4. Drain excess methanol and allow smear to dry.
5. If using SpotTest™ Acridine Orange Stain, hold the dispenser upright with the tip pointing in an outward direction. Using the provided ampule crusher, squeeze gently to crush the glass ampule inside the dispenser. Invert and squeeze slightly to dispense the stain on a per drop basis.

6. Flood the slide with Acridine Orange Stain for 2 minutes.
7. Rinse thoroughly with tap water and allow to dry.
8. Smears may be initially examined at 100X to 400X magnification using a fluorescent microscope. Findings should be confirmed by examination at 1000X with an oil immersion objective.

Results

Bacteria and fungi stain bright orange. The background appears black to yellow green. Human epithelial and inflammatory cells and tissue debris stain pale green to yellow. Activated leukocytes will stain yellow, orange or red depending on the level of activation and the amount of RNA produced. Erythrocytes either do not stain or stain pale green.

Limitations of the Procedure

1. Acridine Orange staining provides presumptive information on the presence and identification of microorganisms in the specimen. Because microorganisms seen in smears, including nonviable organisms, may arise from external sources (i.e., specimen collection devices, slides or water used for rinsing), all positive smears should be confirmed by culture.
2. Approximately 10^4 colony-forming units per ml are required for detection by the Acridine Orange staining method.
3. Acridine orange staining does not distinguish between gram-positive and gram-negative organisms. The gram reaction may be determined by performing the Gram stain procedure directly over the acridine orange stain after removing the immersion oil with xylene.[12]
4. Nuclei or granules from disintegrated, activated leukocytes may resemble cocci at lower magnifications (e.g., 100X-400X). They may be distinguished on the basis of morphology at higher magnifications (e.g., 1000X).
5. Certain types of debris may fluoresce in Acridine Orange stained smears. This debris may be distinguished from microorganisms on the basis of morphology when viewed at higher magnification.

References

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6. **Lauer, B. A., L. B. Reller, and S. Mirrett.** 1981. Comparison of acridine orange and Gram stains for detection of microorganisms in cerebrospinal fluid and other clinical specimens. J. Clin. Microbiol. **14**:201-205.
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9. **Rosendal, S., and A. Valdivieso-Garcia.** 1981. Enumeration of mycoplasmas after acridine orange staining. Appl. Environ. Microbiol. **41**:1000-1002.
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12. **Baron, E. J., and S. M. Finegold.** 1990. Bailey & Scott's diagnostic microbiology, 8th ed. The C. V. Mosby Company, St. Louis, MO.

Packaging

Acridine Orange Stain	1 x 250 ml	3336-75
	6 x 250 ml	3336-76
SpotTest™ Acridine Orange Stain	50 x 0.75 ml	3561-26

Bacto® Gram Stain Sets and Reagents

Gram Stain Set · Gram Stain Set (with Stabilized Iodine) 3-Step Gram Stain Set-S · 3-Step Gram Stain Set-T

Intended Use

Bacto Gram Stain Sets and reagents are used to stain microorganisms from cultures or specimens by the differential Gram method.

Summary and Explanation

The Gram stain was devised in 1884 by Christian Gram[1] in an attempt to differentiate bacterial cells from infected tissue. Although Gram observed what is now called the "Gram reaction," he did not recognize the taxonomic value of his technique.[2]

The Hucker[3] modification of the Gram stain is now used to differentiate intact, morphologically similar bacteria into two groups based on cell color after staining. In addition, cell form, size and structural details are evident. Such preliminary information provides important clues to the type of organism(s) present, the further techniques required

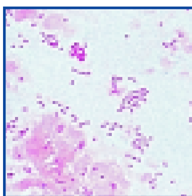
to characterize them, and the therapy to initiate while awaiting test results.

Principles of the Procedure

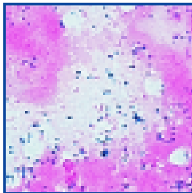
The Gram stain procedure consists of[4,5,6]:

1. Staining a fixed smear with crystal violet;
2. Applying iodine as a mordant;
3. Decolorizing the primary stain with alcohol/acetone; and,
4. Counterstaining with safranin or basic fuchsin.

A crystal violet-iodine complex forms in the protoplast (not the cell wall) of all organisms stained by this procedure. Organisms able to retain this dye complex after decolorization are classified as gram-positive while those that can be decolorized and counterstained are classified as gram-negative.[2,4,5,6]



Positive Blood Culture Bottle
Specimen containing numerous gram-negative rods with shape and size of enteric rods. The culture grew *Klebsiella pneumoniae.*



Ground Beef
Sample containing *E. coli*: H7 and *Staphylococcus aureus.*

Upon disruption or removal of the cell wall, the protoplast of gram-positive (as well as gram-negative) cells can be decolorized and the gram-positive attribute lost. Thus, the mechanism of the Gram stain appears to be related to the presence of an intact cell wall able to act as a barrier to decolorization of the primary stain.

User Quality Control

Run controls daily using 18-24 hour cultures of known gram-positive and gram-negative microorganisms. It is very important that controls be included in each staining run, preferably on the same slide. When performing the Gram stain on a clinical specimen, particularly when the results will be used as a guide to the selection of a therapeutic agent, such a control system furnishes assurance that the iodine solution is providing proper mordant activity and that decolorization was performed properly.

ORGANISM*	ATCC®	EXPECTED RESULTS
Staphylococcus aureus	25923	gram-positive cocci
Escherichia coli	25922	gram-negative rods

* Available as Bactrol™ Disks.

Generally, the cell wall is nonselectively permeable. It is theorized that during the Gram stain procedure, the cell wall of gram-positive cells is dehydrated by the alcohol in the decolorizer and loses permeability, thus retaining the primary stain. However, the cell wall of gram-negative cells has a higher lipid content and becomes more permeable when treated with alcohol, resulting in loss of the primary stain.

The principles of the 3-Step Gram Stain procedure are identical to the 4-step procedure described above. However, the decolorizing and counterstaining steps have been combined into one reagent.

The molecular basis for the Gram stain has not yet been determined.

Formula

Reagents are provided in two sizes, a 250 ml plastic dispensing bottle with a dropper cap and a one-gallon container with a dispensing tap.

Standardization may include adjustment to meet performance specifications.

3329-Gram Crystal Violet
PRIMARY STAIN
Aqueous solution of Crystal Violet.

3331-Gram Iodine
MORDANT
(Working solution prepared from Gram Diluent and Gram Iodine 100X)

Iodine Crystals	3.3	g
Potassium Iodide	6.6	g
Distilled Water	1	liter

3342-Stabilized Gram Iodine
MORDANT

Polyvinylpyrrolidone-Iodine Complex	100	g
Potassium Iodide	19	g
Distilled Water	1	liter

3330-Gram Decolorizer
DECOLORIZER

Acetone	250	ml
Isopropanol	750	ml

3332-Gram Safranin
COUNTERSTAIN

Safranin O Powder (pure dye)	4	g
Denatured Alcohol	200	ml
Distilled Water	800	ml

3343-Gram Basic Fuchsin
COUNTERSTAIN

Basic Fuchsin	0.08	g
Phenol	2.6	g
Isopropyl Alcohol	4.5	ml
Distilled Water	993	ml

3335-3-Step Gram Safranin-S
DECOLORIZER/COUNTERSTAIN
Alcohol-based solution of safranin.*

3341-3-Step Gram Safranin-T
DECOLORIZER/COUNTERSTAIN
Alcohol-based solution of safranin.*

* Patent Pending

Precautions

1. For In Vitro Diagnostic Use.
2. **3329-Gram Crystal Violet**
   IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe spray. Wear suitable protective clothing. Keep container tightly closed.

   3331-Gram Iodine 100X
   HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe fumes. Wear suitable protective clothing. Keep container tightly closed.

   3342-Stabilized Gram Iodine
   HARMFUL IN CONTACT WITH SKIN. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe fumes. Wear suitable protective clothing. Keep container tightly closed.

   3330 - Bacto Gram Decolorizer
   HIGHLY FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist or vapor. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

   3332 - Bacto Gram Safranin
   FLAMMABLE.[EC] HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe vapor. Wear suitable protective clothing. Keep container tightly closed.

   3335 - Bacto 3-Step Gram Safranin-S
   HIGHLY FLAMMABLE. HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

   3341 - Bacto 3-Step Gram Safranin-T
   HIGHLY FLAMMABLE. HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

   FIRST AID:

   In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

   Gram Iodine 100X: Take off immediately all contaminated clothing.

   After contact with skin, wash immediately with plenty of water.

   If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice.

   If swallowed seek medical advice immediately and show this container or label.
3. Studies demonstrate that the traditional Gram Iodine working solution (Gram Iodine 100X dissolved in Gram Diluent) is relatively unstable and may cause variability in the Gram stain when sufficient iodine is no longer available to the solution. Protect the iodine solution from undue exposure to air and heat. Include controls in all staining runs or at least once daily (see USER QUALITY CONTROL) to ensure that the solution is providing proper mordant activity.

Storage

Store Gram Stain reagents at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed.

Use the traditional Gram Iodine working solution within three months of preparation, not exceeding the Expiry of either component.

Specimen Collection and Preparation

1. Apply the test specimen to a clean glass slide in a manner that will yield a thin, uniform smear. Emulsify colonies from an 18-24 hour culture in saline to obtain the proper density.
2. Allow the smear to air dry.
3. Fix the smear to the slide using one of the following techniques:
   A. Heat fix by passing the slide through a low flame 2-3 times. Cool the slide to room temperature before staining. NOTE: Do not overheat the slide; excessive heating will cause atypical staining.
   B. Methanol fix[6,7] the slide by flooding with absolute methanol for 1-2 minutes and rinse with tap water before staining. NOTE: For proper fixation, store absolute methanol in a brown screw-capped bottle and replenish the working supply every two weeks.

Reagent Preparation

Prepare the traditional Gram Iodine working solution by adding an entire 2.5 ml ampule of Gram Iodine 100X to 250 ml Gram Diluent or an entire 40 ml vial of Gram Iodine 100X to 1 gallon of Gram Diluent; mix thoroughly.

4-Step Staining Procedure[4]

Materials Provided

4-Step Technique

Gram Crystal Violet
Gram Iodine or Bacto Stabilized Gram Iodine
Gram Decolorizer
Gram Safranin or Bacto Gram Basic Fuchsin

Materials Required but not Provided

Microscope slides
Bunsen burner or methanol
Bacteriological loop

Swabs
Blotting paper
Microscope with oil immersion lens
Bactrol™ Gram Slide
Bactrol™ Disks

1. Flood the fixed smear with primary stain (Gram Crystal Violet) and stain for 1 minute.
2. Remove the primary stain by gently washing with cold tap water.
3. Flood the slide with mordant (either Gram Iodine or Stabilized Gram Iodine) and retain on the slide for 1 minute.
4. Remove the mordant by gently washing with tap water.
5. Decolorize (Gram Decolorizer) until solvent running from the slide is colorless (30-60 seconds).
6. Wash the slide gently in cold tap water.
7. Flood the slide with counterstain (either Gram Safranin or Gram Basic Fuchsin) and stain for 30-60 seconds.
8. Wash the slide with cold tap water.
9. Blot with blotting paper or paper towel or allow to air dry.
10. Examine the smear under an oil immersion lens.

3-Step Staining Procedure

Materials Provided

3-Step Stabilized Iodine Technique

Gram Crystal Violet
Stabilized Gram Iodine
3-Step Gram Safranin-S

3-Step Traditional Iodine Technique

Gram Crystal Violet
Gram Iodine
3-Step Gram Safranin-T

Materials Required but not Provided

Microscope slides
Bunsen burner or methanol
Bacteriological loop
Swabs
Blotting paper
Microscope with oil immersion lens
Bactrol™ Gram Slide
Bactrol™ Disks

1. Flood the fixed smear with primary stain (Gram Crystal Violet) and stain for 1 minute.
2. Remove the primary stain by gently washing with cold tap water.
3. Flood the slide with mordant (Stabilized Gram Iodine or Gram Iodine [traditional formulation]) and retain on the slide for 1 minute. (Refer to LIMITATIONS OF THE PROCEDURE, #5.)
4. Wash off the mordant with decolorizer/counterstain (3-Step Gram Safranin-S or 3-Step Gram Safranin-T). (NOTE: Do not wash off iodine with water.) Add more decolorizer/counterstain solution to the slide and stain 20-50 seconds.
5. Remove the decolorizer/counterstain solution by gently washing the slide with cold tap water.
6. Blot with blotting paper or paper towel or allow to air dry.
7. Examine the smear under an oil immersion lens.

Results

REACTION	4-STEP TECHNIQUE USING GRAM SAFRANIN	4-STEP TECHNIQUE USING BASIC FUCHSIN	3-STEP TECHNIQUE USING EITHER GRAM SAFRANIN-S OR GRAM SAFRANIN-T
Gram-positive	Purple-black cells	Bright purple to purple-black cells	Purple-black to purple cells
Gram-negative	Pink to red cells	Bright pink to fuchsia cells	Red-pink to fuchsia cells

Limitations of the Procedure

1. The Gram stain provides preliminary identification information only and is not a substitute for cultural studies of the specimen.
2. Prior treatment with antibacterial drugs may cause gram-positive organisms from a specimen to appear gram-negative.
3. Use of an 18-24 hour culture is advisable for best results since fresh cells have a greater affinity than old cells for most dyes. This is particularly true of many spore formers, which are strongly gram-positive when examined in fresh cultures but which later become gram-variable or gram-negative.
4. The Gram stain reaction, like the acid-fast reaction, is altered by physical disruption of the bacterial cell wall or protoplast. The cell walls of gram-positive bacteria interpose a barrier which prevents leaching of the dye complex from the cytoplasm. Cell walls of gram-negative bacteria contain lipids soluble in organic solvents, which are then free to decolorize the cytoplasm. Therefore, a microorganism that is physically disrupted by excess heating will not react to Gram staining as expected.
5. 3-Step Gram Safranin-S is intended for use with stabilized iodine. 3-Step Gram Safranin-T is intended for use with traditional iodine. Unsatisfactory results may occur if other combinations of iodine and 3-Step Gram Safranin are used.
6. Over time, a fine precipitate may develop in Gram Basic Fuchsin, 3-Step Gram Safranin-S and 3-Step Gram Safranin-T. Product performance will not be affected.

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4. **Bartholomew, J. W.** 1962. Variables influencing results, and the precise definition of steps in gram staining as a means of standardizing the results obtained. Stain Technol. **37**:139-155.
5. **Kruczak-Filipov, P., and R. G. Shively.** 1992. Gram Stain procedure, p. 1.5.1-1.5.18. *In* H.D. Isenberg (ed.), Clinical Microbiology Procedures Handbook, vol. 1. American Society for Microbiology, Washington, D.C.
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7. **Mangels, J. I., M. E. Cox, and L. H. Lindley.** 1984. Methanol fixation. An alternative to heat-fixation of smear. Diag. Microbiol. Infect. Dis. **2**:129-137.

Packaging

Gram Crystal Violet	6 x 250 ml	3329-76
PRIMARY STAIN	1 gallon	3329-83
Gram Iodine	6 x 250 ml	3331-76
MORDANT	1 gallon	3331-83
Stabilized Gram Iodine	6 x 250 ml	3342-76
MORDANT	1 gallon	3342-83
Gram Decolorizer	6 x 250 ml	3330-76
DECOLORIZER	1 gallon	3330-83
Gram Safranin	6 x 250 ml	3332-76
COUNTERSTAIN	1 gallon	3332-83
Gram Basic Fuchsin	6 x 250 ml	3343-76
COUNTERSTAIN	1 gallon	3343-83
3-Step Gram Safranin-S	6 x 250 ml	3335-76
DECOLORIZER/COUNTERSTAIN	1 gallon	3335-83
3-Step Gram Safranin-T	6 x 250 ml	3341-76
DECOLORIZER/COUNTERSTAIN	1 gallon	3341-83

Gram Stain Set		4 x 250 ml	3328-32
Contents:	Gram Crystal Violet	250 ml	
	Gram Iodine	250 ml	
	Gram Decolorizer	250 ml	
	Gram Safranin	250 ml	
Gram Stain Set (with Stabilized Iodine)		4 x 250 ml	3338-32
Contents:	Gram Crystal Violet	250 ml	
	Stabilized Gram Iodine	250 ml	
	Gram Decolorizer	250 ml	
	Gram Safranin	250 ml	
3-Step Gram Stain Set-S		3 x 250 ml	3334-3
Contents:	Gram Crystal Violet	250 ml	
	Stabilized Gram Iodine	250 ml	
	3-Step Gram Safranin-S	250 ml	
3-Step Gram Stain Set-T		3 x 250 ml	3337-32
Contents:	Gram Crystal Violet	250 ml	
	Gram Iodine	250 ml	
	3-Step Gram Safranin-T	250 ml	
Bactrol™ Gram Slide		50 slides	3140-26

Bacto® TB Stain Sets and Reagents

TB Stain Set K · TB Stain Set ZN · TB Fluorescent Stain Set M TB Fluorescent Stain Set T

Intended Use

Bacto TB Stain Sets are used to stain smears prepared from specimens suspected of containing mycobacteria for early presumptive diagnosis of mycobacterial infection.

Also Known As

TB Stain Set K is also known as the Kinyoun Stain.

TB Stain Set ZN is also known as the Ziehl-Neelsen Stain.

TB Fluorescent Stain Set M is also known as the Morse Stain.

TB Fluorescent Stain Set T is also known as the Truant Stain.

Summary and Explanation

The microscopic staining technique is one of the earliest methods devised for detecting the tubercle bacillus and it remains a standard procedure.[1-7] The unique acid-fast characteristic of mycobacteria makes the staining technique valuable in early presumptive diagnosis, and provides information about the number of acid-fast bacilli present. Fluorescent microscopy offers many advantages over classic methods for detecting mycobacteria because of its speed and simplicity, the ease of examining the slide, and the reliability and superiority of the method.[8]

TB Stain Set K uses the Kinyoun (cold) acid-fast procedure described by Kinyoun.[4,9]

TB Stain Set ZN uses the Ziehl-Neelsen (hot) acid-fast procedure described by Kubica and Dye.[4,10]

TB Fluorescent Stain Set M uses the auramine O acid-fast fluorescent procedure described by Morse, Blair, Weiser and Sproat.[4,11]

TB Fluorescent Stain Set T uses the acid-fast fluorescent procedure described by Truant, Brett and Thomas.[4,12]

Principles of the Procedure

The lipid content of the cell wall of acid fast bacilli makes staining of these organisms difficult. In acid fast stains, the phenol allows penetration of the primary stain, even after exposure to acid-alcohol decolorizers. For an organism to be termed acid fast, it must resist decolorizing by acid-alcohol. A counterstain is then used to emphasize the stained organisms, so they may be easily seen microscopically.

When using Stain Set K, acid fast bacilli (AFB) appear red against a green background if Brilliant Green K is used as the counterstain or red against a blue background if Methylene Blue is the counterstain.

When using Stain Set ZN, AFB appear red against a blue background because Methylene Blue is used as the counterstain.

When using Stain Set M, AFB have a bright yellow-green fluorescence.

When using Stain Set T, AFB have a reddish-orange fluorescence.

Formula

3326-TB Stain Set K

Formulas per Liter

3321-TB Carbolfuchsin KF

Basic Fuchsin	15 g
Phenol USP	45 g
Isopropanol	200 ml
Ethanol	50 ml
Distilled Water	750 ml

3318-TB Decolorizer

Hydrochloric Acid .... 30 ml
Denatured Ethanol .... 970 ml

3327-TB Brilliant Green K

Brilliant Green .... 2 g
Sodium Hydroxide .... 0.02 g
Distilled Water .... 1000 ml

User Quality Control

It is recommended that a positive and negative control slide, such as Bactrol™ TB Slide, be included with each batch of slides stained with acid fast stains.

Identity Specifications

3313-TB Carbolfuchsin ZN
Appearance: Reddish-purple suspension with no visible precipitate.

3314-TB Decolorizer TM
Appearance: Colorless, clear suspension.

3315-TB Potassium Permanganate
Appearance: Purple solution.

3316-TB Auramine M
Appearance: Yellow suspension.

3317-TB Auramine-Rhodamine T
Appearance: Red, viscous solution.

3318-TB Decolorizer
Appearance: Colorless, clear solution.

3319-TB Methylene Blue
Appearance: Blue solution with no visible precipitation.

3321-TB Carbolfuchsin KF
Appearance: Reddish purple suspension.

3327-TB Brilliant Green
Appearance: Green solution.

Stain Value

Stain Bactrol™ TB Slides (3139) using the appropriate TB stain procedure. Examine slides using a light or fluorescent microscope at a total magnification of 1000X (oil immersion).

ORGANISM	ATCC®	TB STAIN SET K USING TB BRILLIANT GREEN	TB STAIN SET K USING TB METHYLENE BLUE	TB STAIN SET ZN
Positive Control				
M. tuberculosis H37 Ra	25177	Dark pink to red	Dark pink to red	Dark pink to red
Negative Control				
S. aureus	25923	Green	Blue	Blue
K. pneumoniae	13883	Green	Blue	Blue

ORGANISM	ATCC®	TB FLUORESCENT STAIN SET M	TB FLUORESCENT STAIN SET T
Positive Control			
M. tuberculosis H37 Ra	25177	Bright yellow-green fluorescence	Reddish, orange fluorescence
Negative Control			
S. aureus	25923	No fluorescence	No florescence
K. pneumoniae	13883	No fluorescence	No florescence

3324-TB Stain Set ZN

Formulas per Liter

3313-TB Carbolfuchsin ZN

Basic Fuchsin .... 1.7 g
Phenol USP .... 50 g
Isopropanol .... 95 ml
Distilled Water .... 905 ml

3318-TB Decolorizer

Hydrochloric Acid .... 30 ml
Denatured Ethanol .... 970 ml

3319-TB Methylene Blue

Methylene Blue USP .... 2.4 g
Ethanol .... 300 ml
Distilled Water .... 700 ml

3323-TB Fluorescent Stain Set M

Formulas per Liter

3316-TB Auramine M

Auramine O .... 2 g
Phenol USP .... 4 g
Glycerine USP .... 100 ml
Isopropanol .... 250 ml
Distilled Water .... 650 ml

3314-TB Decolorizer TM

Hydrochloric Acid .... 5 ml
Isopropanol .... 700 ml
Distilled Water .... 300 ml

3315-TB Potassium Permanganate

Potassium Permanganate .... 5 g
Distilled Water .... 1000 ml

3325-TB Fluorescent Stain Set T

Formulas per Liter

3317-TB Auramine-Rhodamine T

Auramine O .... 12 g
Rhodamine B .... 6 g
Phenol USP .... 80 g
Glycerine USP .... 600 ml
Isopropanol .... 140 ml
Distilled Water .... 260 ml

3314-TB Decolorizer TM

Hydrochloric Acid .... 5 ml
Isopropanol .... 700 ml
Distilled Water .... 300 ml

3315-TB Potassium Permanganate

Potassium Permanganate .... 5 g
Distilled Water .... 1000 ml

Precautions

1. For In Vitro Diagnostic Use.
2. **3313-TB Carbolfuchsin ZN**

   IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. TOXIC IN CONTACT WITH SKIN AND IF SWALLOWED.[EC] CAUSES BURNS.[EC] POSSIBLE RISK OF IRREVERSIBLE

EFFECTS.[EC] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

3314-TB Decolorizer TM

HIGHLY FLAMMABLE. CAUSES BURNS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3315-TB Potassium Permanganate

IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

3316-TB Auramine M

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3317-TB Auramine-Rhodamine T

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. TOXIC IN CONTACT WITH SKIN AND IF SWALLOWED.[EC] CAUSES BURNS.[EC] POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3318-TB Decolorizer

HIGHLY FLAMMABLE. HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.[US] POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3319-TB Methylene Blue

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL BY INHALATION AND IF SWALLOWED. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe vapors. Wear suitable protective clothing. Keep container tightly closed.

3321-TB Carbolfuchsin KF

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL BY INHALATION AND IF SWALLOWED.[EC] POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

FIRST AID:

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

After contact with skin, wash immediately with plenty of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice.

If swallowed seek medical advice immediately and show this container or label.

Storage

Store TB Stain Sets and reagents at 15-30°C. Reagents that have been removed from the packing carton should be stored in the dark.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TB Stain Set K*
TB Stain Set ZN*
TB Fluorescent Stain Set M*
TB Fluorescent Stain Set T*
Bactrol™ TB Slides

*Individual reagents available separately. See Packaging.

Materials Required but not Provided

Microscope slides–new or cleaned in acid dichromate solution
Staining rack
Microscope with oil immersion lens, OR
Fluorescent microscope
(See #8 of each fluorescent staining procedure for a complete description of the appropriate assembly required.)

Specimen Collection and Preparation

1. Acid fast stains may be performed on any type of clinical specimen suspected of containing mycobacteria.[4-5] Smears from sputum and other respiratory tract secretions are usually made from concentrated specimens. For procedures used in concentrating specimens for acid fast bacilli, please consult appropriate references.[4-7]
2. Apply a thin smear of the specimen directly on a clear microscope slide.
3. Allow smear to air dry.
4. Fix the smear to the slide by passing the slide through a low flame 2-3 times, avoiding excessive heat.

Test Procedure

See appropriate references for specific procedures.

Kinyoun Stain

TB Stain Set K

1. Place slides on a staining rack and flood with TB Carbolfuchsin KF for 4 minutes. Do not heat.
2. Wash gently in running water.
3. Decolorize with TB Decolorizer for 3-5 seconds, or until no more red color appears in washing.
4. Wash gently in running water.
5. Counterstain with either TB Brilliant Green K or TB Methylene Blue (available separately) for 30 seconds.

6. Wash gently in running water.
7. Air dry. If using TB Methylene Blue, dry over gentle heat.

Ziehl-Neelsen Stain

TB Stain Set ZN

1. Place slides on a staining rack and flood with TB Carbolfuchsin ZN. Heat gently to steaming and allow to steam for 5 minutes.
2. Wash gently in running water.
3. Decolorize with TB Decolorizer for 3-5 seconds or until no more red color appears in washing.
4. Wash gently in running water.
5. Counterstain with either TB Methylene Blue or TB Brilliant Green K for 30 seconds.
6. Wash gently in running water.
7. Dry over gentle heat.

Morse Stain

Fluorescent Stain Set M

1. Place slides on a staining rack and flood with TB Auramine M for 15 minutes.
2. Wash gently in running water.
3. Decolorize with TB Decolorizer TM for 30-60 seconds.
4. Wash slides gently in running water.
5. Counterstain with TB Potassium Permanganate for 2 minutes.
6. Wash gently in running water.
7. Air dry.
8. Examine under a microscope fitted, as described by Morse et al.,[11] with an incandescent bulb, a KG 1 heat filter, a 3-4 mm thick BG excitation filter, an ordinary substage condenser and a No. 51 bright field or GG barrier filter.

Truant Stain

TB Fluorescent Stain Set T

1. Place slides on a staining rack and flood with TB Auramine-Rhodamine T that has been thoroughly shaken prior to use. Leave undisturbed for 20-25 minutes at room temperature.
2. Wash gently in running water.
3. Decolorize with TB Decolorizer TM for 2-3 minutes.
4. Wash gently in running tap water.
5. Counterstain with TB Potassium Permanganate for 4-5 minutes.
6. Wash gently in running water.
7. Blot lightly. Dry in air or very gently over a flame.
8. Examine under a microscope fitted, as described by Truant et al.,[13] with 25X objective, an HBO L2 bulb heat filter, a BG 12 primary filter and OG 1 barrier filter.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. A positive staining reaction provides presumptive evidence of the presence of *M. tuberculosis* in the specimen. A negative staining reaction does not necessarily indicate that the specimen will be culturally negative for *M. tuberculosis*. For positive identification of *M. tuberculosis*, cultural methods must be employed.
2. Rapidly growing mycobacteria may retain acid-fast stains to a varying degree. Most rapidly growing mycobacteria will not fluoresce in fluorochrome-stained smears.[4]
3. Organisms other than mycobacteria, such as *Rhodococcus* spp., *Nocardia* spp., *Legionella micdadei*, and the cysts of *Cryptosporidium* spp. and *Isospora* spp., may display various degrees of acid-fastness.[4]
4. When decolorizing with acid-alcohol, avoid under-decolorization. It is difficult to over-decolorize acid-fast organisms.
5. During the counterstaining step with potassium permanganate, timing is critical. Quenching the fluorescing bacilli occurs when counterstaining for a longer period of time.[4]
6. If fluorochrome stained slides cannot be observed immediately, they may be stored at 2-8°C in the dark for up to 24 hours. This is required to prevent fading of the fluorescence.[4]
7. Prolonged counterstaining in non-fluorochrome stains may mask the presence of acid-fast bacilli. Use of brilliant green may help to minimize this problem.[4]

References

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2. **Neelsen, F.** 1883. Ein Casuistischer Beitrag zur Lehre von der Tuberkulose. Centralbl. Med. Wiss. **21**:497-501.
3. **National Tuberculosis Association.** 1961. Diagnostic Standards and Classification of Tuberculosis. National Tuberculosis Association, New York, NY.
4. **Master, R. N.** 1992. Mycobacteriology, p. 3.0.1-3.16.4. *In* Isenberg, H. D. (ed.), Clinical 2microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
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6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
7. **Kent, P. T., and G. P. Kubica.** 1985. Public Health Mycobacteriology: a guide for the Level III laboratory, p. 57-68. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, GA.
8. **Taylor, R. D.** 1966. Modification of the Brown and Brenn Gram Stain for the differential staining of gram-positive and gram-negative bacteria in tissue sections. Am. J. Clin. Pathol. **46**:472-4.
9. **Kinyoun, J. J.** 1915. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. Am. J. Pub. Health. **5**:867-70.
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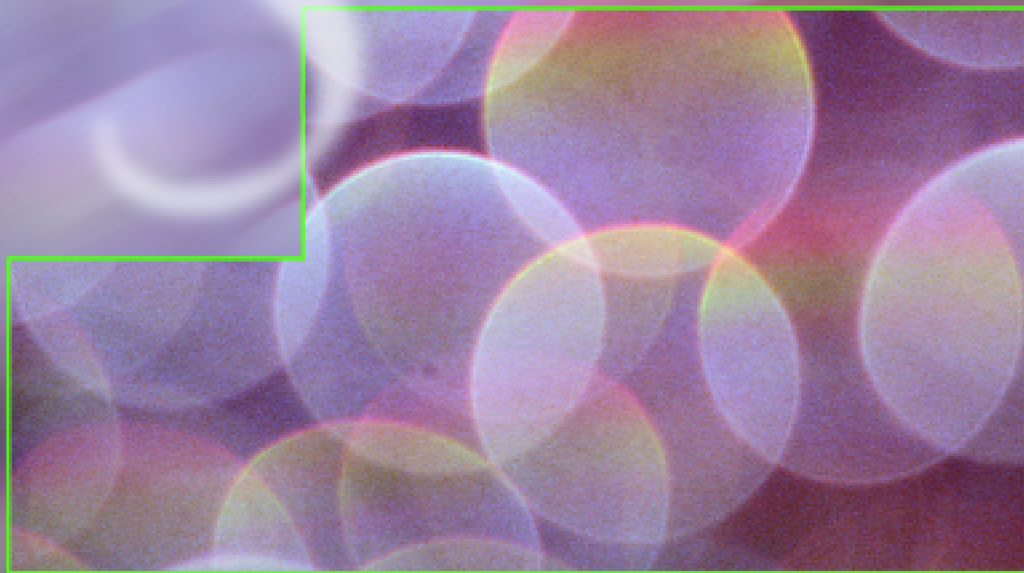
12. **Truant, J. P., W. A. Brett, and W. Thomas.** 1962. Fluorescence microscopy of tubercle bacilli stained with auramine and rhodamine. Bull. Henry Ford Hosp. **10**:287-296.

13. **Willis, H. S., and M. M. Cummings.** 1952. Diagnostic and Experimental Methods in Tuberculosis, 2nd ed. Charles C. Thomas, Springfield, IL.

Packaging

TB Stain Set K	3 x 250 ml	3326-32
Contains:		
TB Carbolfuchsin KF	250 ml	
TB Decolorizer	250 ml	
TB Brilliant Green K	250 ml	
TB Stain Set ZN	3 x 250 ml	3324-32
Contains:		
TB Carbolfuchsin ZN	250 ml	
TB Decolorizer	250 ml	
TB Methylene Blue	250 ml	
TB Fluorescent Stain Set M	3 x 250 ml	3323-32
Contains:		
TB Auramine M	250 ml	
TB Decolorizer TM	250 ml	
TB Potassium Permanganate	250 ml	
TB Fluorescent Stain Set T	3 x 250 ml	3325-32
Contains:		
TB Auramine-Rhodamine T	250 ml	
TB Decolorizer TM	250 ml	
TB Potassium Permanganate	250 ml	
TB Auramine M	6 x 250 ml	3316-76
TB Auramine-Rhodamine T	6 x 250 ml	3317-76
TB Brilliant Green K	6 x 250 ml	3327-76
TB Carbolfuchsin KF	6 x 250 ml	3321-76
TB Carbolfuchsin ZN	6 x 250 ml	3313-76
TB Decolorizer	6 x 250 ml	3318-76
TB Decolorizer TM	6 x 250 ml	3314-76
TB Methylene Blue	6 x 250 ml	3319-76
TB Potassium Permanganate	6 x 250 ml	3315-76
Bactrol™ TB Slides	50 slides	3139-26

Serology and Immunology



Bacto® Bordetella Antigens and Antiserum

Bordetella Pertussis Antiserum · Bordetella Parapertussis Antiserum · Bordetella Pertussis Antigen

Intended Use

Bacto Bordetella Pertussis Antiserum and Bacto Bordetella Parapertussis Antiserum are used in the slide agglutination test for identifying *Bordetella pertussis* and *Bordetella parapertussis*.

Bacto Bordetella Pertussis Antigen is used to demonstrate a positive quality control test in the slide agglutination test.

Summary and Explanation

All members of the genus *Bordetella* are respiratory pathogens of warm-blooded animals. Two species, *B. pertussis* and *B. parapertussis*, are uniquely human pathogens. These organisms adhere to, multiply among and remain localized in the ciliated epithelial cells of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder, less frequently occurring form of the disease.[1] Person-to-person transmission occurs by the aerosol route. Pertussis is a highly contagious disease that, more than 90% of the time, attacks unimmunized populations.[2] Toxin production remains the major distinction between *B. pertussis* and *B. parapertussis*.

Classic pertussis caused by *B. pertussis* occurs in three stages. The first (catarrhal) stage is characterized by nonspecific symptoms similar to a cold or viral infection. The disease is highly communicable during this stage, which lasts 1-2 weeks. During the second (paroxysmal) stage, the cough increases in intensity and frequency. This stage is marked by sudden attacks of severe, repetitive coughing, often culminating with the characteristic whoop. The whooping sound is caused by the rapid inspiration of air after the clearance of mucus-blocked airways.[3] This stage may last 1-4 weeks. The beginning of the convalescent stage is marked by a reduction in frequency and severity of coughing spells. Complete recovery may require weeks or months.

User Quality Control

Identity Specifications

Bordetella Pertussis Antiserum
Bordetella Parapertussis Antiserum
Lyophilized Appearance: Light gold to amber, button to powdered cake.
Rehydrated Appearance: Light gold to amber, clear liquid.

Bordetella Pertussis Antigen
Appearance: Light gray to white suspension, may settle upon standing.

Performance Response

Rehydrate Bordetella Pertussis and Parapertussis Antiserum per label directions. Test as described (see Test Procedure). Bordetella Pertussis Antigen or known positive and negative control cultures must give appropriate reactions.

Despite the availability of an effective whole-cell vaccine, pertussis remains a disease of worldwide distribution because many developing nations do not have the resources for vaccinating their populations.[4] Major outbreaks have occurred even in developed nations such as Great Britain and Sweden. Pertussis is endemic in the United States, with most disease occurring as isolated cases. There has been a shift in the age group affected by the disease. In the past, children in the 1-5 year age group were more prone to pertussis. Since adults do not receive booster vaccinations, children less than one year of age[2] have become more susceptible because of a decrease in passively transferred maternal antibodies.

Bordetella are tiny, gram-negative, strictly aerobic coccobacilli that occur singly or in pairs and may exhibit a bipolar appearance. While some species are motile, *B. pertussis* and *B. parapertussis* are nonmotile. They do not produce acid from carbohydrates. *B. pertussis* will not grow on common blood agar bases or chocolate agar, while *B. parapertussis* will grow on blood agar and sometimes on chocolate agar. Media for primary isolation must include starch, charcoal, ion-exchange resins or a high percentage of blood to inactivate inhibitory substances.[3] *B. pertussis* may be recovered from secretions collected from the posterior nasopharynx, bronchoalveolar lavage and transbronchial specimens.

Principles of the Procedure

Identification of *Bordetella* species includes isolation of the microorganisms, biochemical identification and serological confirmation.

Serological confirmation involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. The *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (has high avidity), and binds strongly (has high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to some other species, heterologous reactions are possible. These are weak in strength or slow in formation. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Homologous reactions are rapid and strong. Heterologous reactions are slow and weak.

Reagents

Bordetella Pertussis and Parapertussis Antisera are lyophilized, polyclonal rabbit antiglobulins containing approximately 0.04% Thimerosal as a preservative. When rehydrated and used as described, each 1 ml vial of Bordetella Pertussis or Parapertussis Antiserum diluted 1:10 contains sufficient reagent for 200 slide tests.

Bordetella Pertussis Antigen is a ready-to-use suspension of killed, whole organisms adjusted to a density approximating two times a McFarland Barium Sulfate Standard No. 3 (9 x 10^8 organisms per ml). Bordetella Pertussis Antigen contains 0.04% Thimerosal. When used as described, each 5 ml vial contains sufficient reagent to perform approximately 140 slide tests.

Because antigen density may vary, it is adjusted to ensure optimum performance when the antigen is standardized with hyperimmune sera obtained from laboratory animals.

Precautions

1. For In Vitro Diagnostic Use.
2. **Bordetella Pertussis Antiserum**
   Bordetella Parapertussis Antisera
   The Packaging of This Product Contains Dry Natural Rubber.
3. Bordetella Pertussis Antigen is not intended for use in the immunization of humans or animals.
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Bordetella Pertussis Antiserum and Bordetella Parapertussis Antiserum at 2-8°C.

Store Bordetella Pertussis Antigen at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bordetella Pertussis Antiserum
Bordetella Parapertussis Antiserum
Bordetella Pertussis Antigen

Materials Required But Not Provided

Agglutination slides
Applicator sticks
Sterile 0.85% NaCl solution
Distilled or deionized water
Inoculating loop

Reagent Preparation

Equilibrate all materials to room temperature before performing the test. Ensure that all glassware and pipettes are clean and free of detergent residues.

Bordetella Pertussis and Parapertussis Antiserum: To rehydrate, add 1 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents. Dilute the rehydrated antiserum 1:10 with sterile 0.85% NaCl solution. Dilute only enough for 1-2 days testing requirements.

Bordetella Pertussis Antigen is ready to use.

Specimen Collection and Preparation

Isolation of *Bordetella* from clinical specimens requires the use of certain media such as Bordet-Gengou Agar. Colonies of *B. pertussis* are very small, white, glistening, convex, entire and usually exhibit tiny zones of hazy hemolysis. Colonies of *B. parapertussis* are usually larger than those of *B. pertussis,* may have a slightly brown color, and do not have a glistening surface. For specific recommendations for culture and identification, consult appropriate references.[3,5] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *Bordetella.* After these criteria are met, serological identification can proceed.

Test Procedure

1. **Bordetella Antiserum**: On an agglutination slide, dispense 2 separate drops (approximately 35 µl, each) of the antiserum to be tested, the first to be used for the test isolate and the second for the positive control.
2. **Negative control**: Dispense 1 drop of sterile 0.85% NaCl solution.
3. **Test isolate**: Transfer a loopful of isolated colony from a solid agar medium to the first drop of antiserum and a second loopful to the negative control (0.85% NaCl solution).
4. **Positive control**: As appropriate, add 1 drop of Bordetella Pertussis Antigen or a small amount of a known *B. pertussis* or *B. parapertussis* culture to the second drop of antiserum.
5. Mix each test and control serum reaction area using separate applicator sticks.
6. Rotate the slide for 1 minute and read for agglutination.

Results

1. Read and record results as follows.
   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.
2. **Positive control**: Should produce 3+ or greater agglutination.
   Negative control: Should produce no agglutination.
   Positive test result: Agglutination of 3+ or greater within 1 minute.

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity as well as morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as a *Bordetella* species.
2. Serological methods alone cannot identify the isolate as *Bordetella*.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or may cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates do occur and will agglutinate spontaneously causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
6. A rehydrated Bordetella Antiserum that is cloudy or develops a precipitate during use should be discarded.

7. Some *Hemophilus* species will grow on *Bordetella* isolation media and may cross-react with *B. pertussis* antisera. Rule out X- and V-factor dependence using Differentiation Disks V, X and VX.[5]
8. Shake the antigen vial well before use to obtain a smooth, uniform suspension. Occasionally, a *Bordetella* suspension may settle out during storage.
9. *Bordetella* antigen will display irreversible autoagglutination if, at anytime during shipment or storage, it is subjected to freezing temperatures. Do not allow to freeze.

References

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Packaging

Bordetella Pertussis Antiserum	1 ml	2309-50
Bordetella Parapertussis Antiserum	1 ml	2310-50
Bordetella Pertussis Antigen	5 ml	2585-56

Bacto® Brucella Antigens and Antisera

Brucella Abortus Antigen (Slide) · Brucella Abortus Antigen (Tube) Brucella Melitensis Antigen (Slide) · Brucella Suis Antigen (Slide) Brucella Abortus Antiserum · Febrile Negative Control

Intended Use

Bacto Brucella Abortus Antigen (Slide) and (Tube), Brucella Melitensis Antigen (Slide) and Brucella Suis Antigen (Slide) are used in the detection of antibodies by the slide and tube agglutination tests (as indicated).

Bacto Brucella Abortus Antiserum is used to demonstrate a positive quality control test reaction in the slide and tube agglutination tests.

Bacto Febrile Negative Control is used to demonstrate a negative quality control test reaction in the slide agglutination test.

Summary and Explanation

Brucellae are intracellular parasites that, upon invasion, produce fever in their host. Consequently, they are often called “Febrile Antigens.” Brucellae also cause localized infection of bone, tissue and organ systems in humans.[1,2] These organisms are intracellular pathogens of the reticuloendothelial system. They form granulomatous masses in various organs.

Brucellosis is the disease state caused by these organisms. The disease has an abrupt onset, usually three to four weeks after exposure. Symptoms of the disease include fever, arthralgia, malaise, chills and sweating. Approximately 70% of patients with acute brucellosis have a tube agglutination titer of 1:160 or greater.[3] Osteomyelitis is the most frequent complication in humans.[4,5]

Most cases of brucellosis are due to exposure to animals, including cattle, sheep, goats and swine, or to laboratory cultures of *Brucella* species.[3] Patient history usually includes exposure to livestock or to meat processing. Routes of infection are nasopharyngeal, gastrointestinal, conjunctival, respiratory and through abraded skin.[5] Sporadic episodes of food-associated brucellosis have been caused by *B. melitensis*.[6,7]

The human immune response to a particular microorganism results in measurable antibody production which, in some cases, can assist in completing the patient’s clinical diagnosis. In blood samples, the antibody titer during the initial phase of the infection (acute) is compared to the antibody titer 7-14 days later (convalescent). A high acute phase antibody titer (1:320) and paired acute and convalescent samples that show an increase in antibody titer are helpful in assisting the diagnosis of brucellosis.

A preliminary test using either the rapid slide test and/or the macroscopic tube test may be performed on the initial serum specimen and reported to the physician at that time. An aliquot of the serum should be transferred to a sterile test tube, sealed tightly, and kept in the freezer. When the second serum is obtained, it should be run in parallel with the original specimen. In this manner, the original serum will serve as a control. Any difference in titer will be more credible because the bias associated with the performance of the test and determining the endpoint will be reduced.

Brucellae are small, nonmotile, nonencapsulated gram-negative coccobacilli. The organisms grow aerobically and their growth is enhanced by incubation in CO_2.

Six species of *Brucella* have been recognized. Among these, *B. abortus* (cattle), *B. suis* (swine), *B. melitensis* (goats and sheep) and *B. canis* (dogs) are infective for humans, although *B. canis* infections in humans are rare.[8]

The rapid slide procedure is a screening test designed to detect agglutinins. The tube test is a confirmatory procedure designed to quantitate febrile agglutinins. It is, therefore, necessary that any positive results obtained in the screening (slide test) of specimens be confirmed by a tube test. The tube agglutination test is used clinically in the United States.[9,10,11]

Certain organisms may share cross-reacting antigens leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in a febrile antibody test procedure, causing low-level antibody titers that may not singly be indicative of disease. Cross reactions between *Brucella* and *Francisella tularensis*, *Yersinia enterocolitica* and *Vibrio cholerae* can occur.

Principles of the Procedure

Agglutination tests involving the use of *Brucella* antigens detect the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum and then adding a standard volume of antigen. The endpoint of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point, reported as a dilution of the serum, is called the patient's antibody "titer."

Reagents

Brucella Abortus Antigen (Slide), Brucella Melitensis Antigen (Slide) and Brucella Suis Antigen (Slide) are ready-to-use, chemically inactivated and stabilized suspensions of *Brucella abortus* 1119-3,[12] *Brucella melitensis* and *Brucella suis*, respectively. The slide antigens contain approximately 2% packed cells and 20% glycerin, as well as 0.5% phenol and approximately 0.002% crystal violet and 0.005% brilliant green as preservatives. When used as described, each 5 ml vial contains sufficient reagent for 20 slide tests.

Brucella Abortus Antigen (Tube) is a ready-to-use suspension of *Brucella abortus* 1119-3[12] adjusted to a density approximating a McFarland Barium Sulfate Standard No. 3 (9×10^8 organisms per ml). Brucella Abortus Antigen (Tube) contains 0.5% phenol as a preservative but does not contain dye. When used as directed, each 25 ml vial contains sufficient reagent for 6 tests.

Because antigen density may vary, density is adjusted to ensure optimum performance when the antigen is standardized with hyperimmune sera obtained from laboratory animals. Variation in antigen color intensity is normal and will not affect the outcome of the test.

Brucella Abortus Antiserum is a lyophilized, polyclonal rabbit antiserum containing approximately 0.04% Thimerosal as a preservative.

Brucella Abortus Antiserum is unabsorbed. Serological cross-reactions occur in unabsorbed sera from *Brucella* species because *B. abortus, B. suis* and *B. melitensis* are antigenically related, containing common A (*abortus*) and M (*melitensis*) substances. (Monospecific sera prepared by absorption produce weak, unstable reagents that make interpretation of agglutination results difficult.)

When rehydrated and used as described, each 3 ml vial of Brucella Abortus Antiserum contains sufficient reagent for 19 slide tests or 30 tube tests.

Febrile Negative Control is a standard protein solution containing approximately 0.04% Thimerosal as a preservative. When used as described, each 3 ml vial contains sufficient reagent for 32 slide tests.

User Quality Control

Identity Specifications

Brucella Abortus Antigen (Slide), Brucella Suis Antigen (Slide), Brucella Melitensis Antigen (Slide)
Appearance: Turquoise, blue-violet suspension.

Brucella Abortus Antigen (Tube)
Appearance: Light gray to white suspension.

Brucella Abortus Antiserum
Lyophilized Appearance: Light gold to amber, button to powdered cake.
Rehydrated Appearance: Light gold to amber, clear liquid.

Febrile Negative Control
Lyophilized Appearance: Colorless to light gold, button to powdered cake.
Rehydrated Appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Brucella Abortus Antiserum and Febrile Negative Control per label directions. Perform the slide or tube agglutination test using Brucella Abortus Antigen (Tube), Brucella Abortus (Slide), Brucella Suis (Slide), or Brucella Melitensis (Slide). Dilute both positive and negative controls in the same proportion as the patient's serum and process in the same manner, following appropriate procedure.

An antigen is considered satisfactory if it does not agglutinate with the negative control and yields a 2+ reaction at a titer of 1:80 or more with the positive control.

Precautions

1. For In Vitro Diagnostic Use.
2. **Brucella Abortus Antiserum**
   The Packaging of This Product Contains Dry Natural Rubber.
3. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[13,14]
4. Brucella Antigens are not intended for use in the immunization of humans or animals.
5. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Brucella Antigens (Slide) and (Tube) at 2-8°C.

Store lyophilized and rehydrated Brucella Abortus Antiserum at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brucella Abortus Antigen (Slide)
Brucella Suis Antigen (Slide)

Brucella Melitensis Antigen (Slide)
Brucella Abortus Antigen (Tube)
Brucella Abortus Antiserum
Febrile Negative Control

Materials Required But Not Provided

Slide Test

Agglutination slides with 5 squares
Applicator sticks
Sterile 0.85% NaCl solution
Serological pipettes, 0.2 ml
Distilled or deionized water

Tube Test

Culture tubes, 12 x 75 mm, and rack
Waterbath, 35-37°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution
Distilled or deionized water

Reagent Preparation

Brucella Abortus Antigen (Slide) and (Tube), Brucella Suis Antigen (Slide) and Brucella Melitensis Antigen (Slide) are ready to use.

Equilibrate all materials to room temperature prior to performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

Brucella Abortus Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to completely dissolve the contents. The rehydrated antiserum is considered a 1:2 working dilution.

Febrile Negative Control: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. After the specimen has clotted, centrifuge to obtain the serum required for the test. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Consult appropriate references for more information on the collection of specimens.[15,16]

Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain below -20°C. Serum specimens must not be heated; heat may inactivate or destroy certain antibodies.

Slide Test

Use the slide test only as a screening test. Confirm positive results with the tube test.

In some cases of brucellosis, sera may display a prozone reaction, the inability of an antigen to react at higher serum antibody concentrations. It is advisable to run all 5 serum dilutions of the rapid slide test, rather than just one dilution, to eliminate the possibility of missing positive reactions due to the prozone phenomenon.

1. **Test serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of each test serum into a row of squares on the agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Brucella Abortus Antiserum into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Antigen**: Shake the vial of Brucella Antigen (Slide) well to ensure a smooth, uniform suspension. Dispense 1 drop (35 μl) of antigen in each drop of test serum, positive control and negative control.
5. Mix the rows of test and control serum, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond to tube dilutions of 1:20, 1:40, 1:80, 1:160 and 1:320, respectively.

Tube Test

1. In a rack, prepare a row of 8 culture tubes (12 x 75 ml) for each test serum, including a positive control row for the Brucella Abortus Antiserum and an antigen control row for the Febrile Negative Control Serum.
2. Dispense 0.9 ml of sterile 0.85% NaCl solution in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, dispense 0.1 ml of serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Proceed in like manner for each serum to be tested. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
4. **Positive control**: Using a 1 ml serological pipette, dispense 0.1 ml of Brucella Abortus Antiserum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
5. **Antigen Control**: Shake the vial of Brucella Abortus Antigen (Tube) to ensure a smooth, uniform suspension. Add 0.5 ml of antigen to all 8 tubes in each row and shake the rack to mix the suspensions.
6. Final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
7. Incubate in a waterbath at 35-37°C for 48 ± 3 hours.
8. Remove from the waterbath. Avoid excessive shaking before reading the reactions, either when the tubes are in the waterbath or when removing them from the waterbath.
9. Read and record results.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.

2. **Positive control**: Should produce 2+ or greater agglutination at a 1:80 dilution.

   Negative control–Rapid Slide Test, only: Should produce no agglutination.

   Antigen control–Macroscopic Tube Test, only: Should show no agglutination in tube #8 of each row.

   If results for either the positive or the negative control are not as specified, the test is invalid and results cannot be reported.

   Test serum: The titer is the highest dilution that shows 2+ agglutination.

   Refer to Table 1 and Table 2 for examples of test reactions.
3. The Rapid Slide Test is a screening test, only; results must be confirmed using the Macroscopic Tube Test.

Table 1. Sample Rapid Slide Test reactions.

SERUM (ml)	CORRELATED TUBE DILUTION	REACTIONS		
		SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
0.08	1:20	3+	4+	4+
0.04	1:40	2+	4+	3+
0.02	1:80	1+	3+	2+
0.01	1:160	–	3+	+
0.005	1:320	–	1+	–
Serum titer		**1:40**	**1:160**	**1:80**

Table 2. Sample Macroscopic Tube Test reactions.

SERUM DILUTION	REACTIONS		
	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
1:20	4+	3+	4+
1:40	4+	2+	4+
1:80	3+	1+	4+
1:160	2+	–	4+
1:320	1+	–	3+
1:640	–	–	2+
1:1280	–	–	1+
Serum titer	**1:160**	**1:40**	**1:640**

Interpretation

For a single serum specimen, a titer of 1:80 is a weak positive that suggests infection, but not necessarily a recent infection.[3,17]

A two-dilution increase in the titer of paired serum specimens (from the acute to the convalescent serum) is significant and suggests infection. A one-dilution difference is within the limits of laboratory error.

Past history in the use of *Brucella* suspensions has produced a pattern of titers that are considered "significant". A titer of 1:80 is considered a weakly positive result while most patients with acute undulant fever demonstrate a titer of 1:160 or greater.

Limitations of the Procedure[18,19]

1. The slide test is intended for screening only and results should be confirmed by the tube test. Slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all 5 slide test serum dilutions should be run.

   The detection of antibodies in serum specimens may complete the clinical picture of brucellosis. However, isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician based on patient history, physical examination and data from all laboratory tests.
3. The accuracy and precision of the tests can be affected not only by test conditions but also by the subjectivity of the person reading the endpoint.
4. Cross-reactions may occur due to antigenic similarities to other organisms. A definite serological relationship exists between *Brucella* and *Francisella tularensis*. Cross-reactions may also occur between *Brucella*-positive sera and *Proteus* OX19 antigen, *Vibrio cholerae* or *Yersinia enterocolitica* serotype 9.[20]
5. While a single serum specimen showing a positive reaction at a 1:80 dilution suggests infection, it is not diagnostic. An antibody titer greater than 1:160 may occur in healthy individuals with a past history of the disease.
6. To test for a significant rise in antibody titer, at least two specimens are necessary, an acute specimen obtained at the time of initial symptoms and a convalescent specimen obtained 7 to 14 days later. A two-dilution increase in titer is significant and suggests infection.
7. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
8. Exposure to temperatures below 2°C can cause autoagglutination. Antigens must be smooth, uniform suspensions. Examine antigen vials for agglutination before use. Agglutinated suspensions are not usable and should be discarded.
9. Adhering to the recommended time and temperature of incubation is important when performing the tube test. For best results, locate the waterbath in an area free of mechanical vibration.
10. Serum specimens from patients suffering from acute brucellosis demonstrate little or no antibody titer during the first 10 days of the disease.
11. Serological interpretation of an agglutinin titer in vaccinated individuals should be avoided since antibody levels may persist for years.
12. Individuals who have recovered from brucellosis may demonstrate a nonspecific agglutinin response upon infection with an etiological agent of a heterologous febrile species.

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Packaging

Brucella Abortus Antigen (Slide)	5 ml	2909-56
Brucella Abortus Antigen (Tube)	25 ml	2466-65
Brucella Melitensis Antigen (Slide)	5 ml	2916-56
Brucella Suis Antigen (Slide)	5 ml	2915-56
Brucella Abortus Antiserum	3 ml	2871-47
Febrile Negative Control	3 ml	3239-56

Bacto® Candida Albicans Antiserum

Intended Use

Bacto Candida Albicans Antiserum is used in the slide agglutination test for identifying *Candida albicans.*

Summary and Explanation

Candida albicans is an opportunistic pathogen. Infection with this organism will usually arise from an endogenous source in a compromised host. Candidiasis caused by *C. albicans* presents as superficial infections of the skin, oral thrush, systemic and disseminated infections involving most internal organs, and mucocutaneous candidiasis.[1] Vaginitis caused by *C. albicans* is the most common type of yeast infection.

C. albicans, a saprophyte, appears in large numbers throughout the oral-gastrointestinal tract of many warm-blooded vertebrates.[1] It is rarely isolated from normal skin. Person-to-person transmission of candidiasis can occur.

Candida albicans appears to possess many virulence attributes that may promote successful parasitism. These attributes include rapid germination upon seeding tissue from the bloodstream,[3] protease production,[4] complement protein-binding receptor,[5,6] surface variation and hydrophobicity.[7]

Candida albicans will grow on Sabauroud Dextrose Agar as white to cream-colored, butyrous colonies. *C. albicans* can be isolated from blood agar as a colony with short marginal extensions. Microscopically, *C. albicans* produces budding yeast cells, pseudohyphae or true hyphae. The organism may be identified by the production of germ tubes or chlamydospores. Identification of *Candida albicans* includes both biochemical and serological confirmation.[8]

Principles of the Procedure

Serological confirmation requires that the microorganism (antigen) react with its corresponding antibody. This in vitro reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (has high avidity), and bonds strongly (has high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to another species, heterologous reactions are possible. These are weak in strength or slow in formation. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Agglutination of the somatic antigen in the slide test appears as a firm granular clumping. Homologous reactions occur rapidly and are strong (3+). Heterologous reactions are slow and weak.

Reagents

Candida Albicans Antiserum is a lyophilized, polyclonal rabbit antiserum containing approximately 0.04% Thimerosal as a preservative.

When rehydrated and used as described, each 3 ml vial contains sufficient Candida Albicans Antiserum for 60 tests.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Candida Albicans Antiserum at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Candida Albicans Antiserum

Materials Required But Not Provided

Agglutination slides
Applicator sticks
0.85% Sodium Chloride
Inoculating loop

User Quality Control

Identity Specifications

Candida Albicans Antiserum

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

Performance Response

Rehydrate Candida Albicans Antiserum per label directions. Perform the slide agglutination test using appropriate positive and negative control cultures. The negative control should show no agglutination. Homologous cultures should give a 3+ or greater agglutination.

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Candida Albicans Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to dissolve the contents completely. The rehydrated antiserum is considered a 1:2 working dilution.

Specimen Collection and Preparation

Candida albicans can be recovered on Tryptic Soy Agar with 5% Sheep Blood, Sabouraud Dextrose Agar or Brain Heart Infusion Agar. For specific recommendations on isolation of *Candida albicans* from clinical specimens, consult appropriate references.[9,10,11]

Test Procedure

1. Culture the test organism on Sabouraud Dextrose Agar at room temperature.
2. **Candida Albicans Antiserum**: Dispense one drop at one end of a microscope slide.
3. **0.85% NaCl solution**: Dispense one drop at the other end of the same slide.
4. **Test organism**: Place a partial loopful of a smooth homologous culture between the drops of antiserum and NaCl solution but not in direct contact with either.
5. Using an applicator stick, suspend the culture in the drop of NaCl solution and check for autoagglutination. If there is no autoagglutination, mix the culture suspension with the drop of antiserum.
6. Rotate the slide by hand for one minute and read immediately for agglutination. Record results.

Results

1. Read and record results as follows.

   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.

3. **Negative control**: Should produce no agglutination. If agglutination occurs, the culture is rough and cannot be tested. Subculture to a non-inhibitory medium, incubate and test the organism again.
4. **Test isolate**: 3+ or greater agglutination is a positive result.
5. Partial (less than 3+) or delayed agglutination should be considered negative.

Limitations of the Procedure

1. Serological techniques employing Candida Albicans Antiserum for the identification of *Candida albicans* serve as corroborative evidence in the determination of the organism as the etiological agent of the disease. Final identification cannot be made without consideration of morphological, serological, and biochemical characterization.
2. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.

3. Rough culture isolates do occur and will agglutinate spontaneously, causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
4. Agglutination reactions of 3+ or greater in the slide test are interpreted as positive reactions. Cross-reactions resulting in a 1+ or 2+ agglutination are likely since somatic antigens are shared among such organisms as *Candida tropicalis*, *Candida kefyr* and *Candida stellatoidea*.
5. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
6. Discard any Candida Albicans Antiserum that is cloudy or has a precipitate after rehydration or storage.

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Packaging

Candida Albicans Antiserum	3 ml	2281-47

Bacto® Coagulase Plasma
Bacto Coagulase Plasma EDTA

Intended Use

Bacto Coagulase Plasma[1] and Bacto Coagulase Plasma EDTA[1-8] are used for detecting coagulase activity by staphylococci.

Bacto Coagulase Plasma is used for detecting the production of germ tubes by *Candida albicans*.[2]

Summary and Explanation

Coagulase Detection

Identification of staphylococci is based on microscopic examination, colonial morphology, and cultural and biochemical characteristics. Staphylococci associated with acute infection (*S. aureus* in humans and *S. intermedius* and *S. hyicus* in animals) can clot plasma. The most widely used and generally accepted criterion for identification of these pathogenic organisms is based on the presence of the enzyme coagulase.[1] The ability of *Staphylococcus* to produce coagulase was first reported by Loeb[9] in 1903. Coagulase binds plasma fibrinogen, causing the organisms to agglutinate or plasma to clot. Two different forms of coagulase can be produced, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase, also known as clumping factor, remains attached to the cell wall of the organism. The tube test can detect the presence of both bound and free coagulase. The slide test can detect only bound coagulase.[10] Isolates that do not produce clumping factor must be tested for the ability to produce extracellular coagulase (free coagulase).

The tube test has traditionally been the standard in determining coagulase activity. The slide test is unreliable in the identification of some strains of oxacillin-resistant *S. aureus*.[11,12] False-positive results are sometimes obtained with the slide test when testing *S. saprophyticus*,[13] *S. schleiferi*, *S. lugdunensis*, *S. intermedius*,[4] *S. hyicus*[3] and micrococci.[11,14] In addition, colonies used for testing must not be picked from media containing high concentrations of salt (for example, mannitol-salt agar), because autoagglutination and false-positive results may occur.[1] Slide tests must be read quickly, because false-positive results may appear with reaction times longer than 10 seconds. Isolates that autoagglutinate cannot be reliably tested with the slide coagulase method. Finally, 10-15% of *S. aureus* strains may yield a negative result, which requires that the isolates be reexamined by the tube test. Coagulase Plasma and Coagulase Plasma EDTA are recommended for performing the tube coagulase test. The inoculum used for testing must be pure because a contaminant may produce false results after prolonged incubation. For the coagulase test, Coagulase Plasma EDTA is superior to citrated plasma because citrate-utilizing organisms such as *Pseudomonas* species, *Serratia marcescens*, *Enterococcus faecalis* and strains of *Streptococcus* will clot citrated plasma in 18 hours.[15]

Germ Tube Development

C. albicans is usually associated with an animal host. It appears in large numbers as a saprophyte throughout the oral-gastrointestinal tract

of many warm-blooded vertebrates.[16] It is rarely isolated from normal skin. Person-to-person transmission of candidiasis can occur. Usually, candidiasis caused by *C. albicans* is endogenous in origin and develops with stress or debilitation of the host.[16]

C. albicans is the species most commonly isolated from patients with nearly all forms of candidiasis.[17] This organism is an opportunistic pathogen and appears to possess many virulence attributes that may promote successful parasitism. These attributes include rapid germination upon seeding tissue from the bloodstream,[18] protease production,[19] complement protein-binding receptor,[20,21] and surface variation and hydrophobicity.[22]

C. albicans will grow on Sabouraud Dextrose Agar as white to cream colored, creamy colonies. It can be isolated from blood agar as a colony with short marginal extensions. Microscopically, *C. albicans* produces budding yeast cells, pseudohyphae or true hyphae.

One of the simplest and most valuable tests for the rapid presumptive identification of *C. albicans* is the germ tube test.[23] Smith and Elliott recommended the use of rabbit coagulase plasma.[2] The test is considered presumptive because not all isolates of *C. albicans* will be germ tube-positive and false positives may be obtained despite well-trained staff.[24] Ferrigno, Ramirez and Robison recommended testing for germ tube production with citrated plasma.[25]

User Quality Control

Identity Specifications

Coagulase Plasma

Lyophilized Appearance: Off-white to cream colored, dried button or fluffy powder.

Rehydrated Appearance: Off-white to cream to light rose colored, opaque liquid.

Coagulase Plasma EDTA

Lyophilized Appearance: Off-white to cream colored, dried button or fluffy powder.

Rehydrated Appearance: Off-white to cream to light rose colored, opaque liquid.

Performance Response

Rehydrate Coagulase Plasma or Coagulase Plasma EDTA per label directions. Perform the Coagulase Test or the Germ Tube Test procedure as described (see Test Procedure).

ORGANISM	ATCC®	COAGULASE TEST	GERM TUBE DEVELOPMENT
Staphylococcus aureus	25923*	Clot in tube	–
Staphylococcus aureus	3647	Clot in tube	–
Staphylococcus epidermidis	12228*	No clot in tube	–
Staphylococcus saprophyticus	15305	No clot in tube	–
Candida albicans	18804	–	Germ tube development
Candida tropicalis	750	–	No germ tube development

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Coagulase Detection

S. aureus produces two types of coagulase, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase, also known as the clumping factor, remains attached to the cell wall of the organism.

In the tube test, free coagulase liberated from the cell acts on prothrombin in the coagulase plasma to give a thrombin-like product. This product then acts on fibrinogen to form a fibrin clot.[3]

The tube test is performed by mixing an overnight broth culture or colonies from a noninhibitory agar plate into a tube of rehydrated coagulase plasma. The tube is incubated at 37°C. The formation of a clot in the plasma indicates coagulase production.

Germ Tube Development

The germ tube test involves suspending suspected colonies of yeast in a tube of Coagulase Plasma. The tube is incubated at 37°C for 2-4 hours. The cells are then observed microscopically for short, hyphal extensions from the yeast cells called germ tubes. Germ tubes are easily differentiated from blastoconidial germination; germ tubes have no constriction at their juncture with the yeast cell while blastoconidial germination does produce a constriction. *C. albicans* usually produces germ tubes under specified test conditions within 2 hours. Other species of *Candida* do not produce germ tubes, except for an occasional isolate of *Candida tropicalis*.[4]

Reagents

Coagulase Plasma is lyophilized rabbit plasma to which sodium citrate has been added as the anticoagulant.

Coagulase Plasma EDTA is lyophilized rabbit plasma to which EDTA (ethylenediaminetetraacetic acid) has been added as the anticoagulant. EDTA is not utilized by bacteria. Coagulase Plasma EDTA does not give false-positive reactions with bacteria that utilize citrate.

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store unopened Coagulase Plasma and Coagulase Plasma EDTA at 2-8°C.

Store reconstituted plasma at 2-8°C for up to 5 days, or aliquot in 0.5 ml amounts, freeze promptly and store at -20°C for up to 30 days. Do not thaw and refreeze.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Coagulase Plasma
Coagulase Plasma EDTA

Materials Required But Not Provided

Bacteriological inoculating loop
Sterile 1 ml pipettes
Sterile Pasteur pipettes
Sterile serological pipettes, 1, 5, and 10 ml
Incubator (37°C)
Sterile distilled or deionized water
Culture tubes, 12 x 75 mm
Timer
Waterbath (35-37°C)
BHI broth or noninhibitory agar (Coagulase Detection)
Sabouraud Dextrose Agar (Germ Tube Development)

Reagent Preparation

Rehydrate Coagulase Plasma and Coagulase Plasma EDTA by adding sterile distilled or deionized water to the vial as indicated below. Mix by gentle end-over-end rotation of the vial.

PRODUCT SIZE	STERILE DISTILLED WATER	APPROXIMATE NUMBER OF TESTS
3 ml	3 ml	6
15 ml	15 ml	30
25 ml	25 ml	50

Specimen Collection and Preparation

1. Collect specimens or samples in sterile containers or with sterile swabs and transport immediately to the laboratory according to recommended guidelines.[1,3-8]
2. Process each specimen using procedures appropriate for that sample.[1,3-8]

Coagulase Detection

1. Obtain a pure culture of the organism to be tested. Select well-isolated colonies.
2. Determine that the test culture has characteristics of *S. aureus* as listed below. Consult appropriate references for further identification of *S. aureus*.[1,3-8]

   Morphology (media dependent):

Blood Agar Base w/5% Sheep Blood	Opaque, yellow to orange, with hemolysis.
DNase Test Agar w/Methyl Green	Clearing of green dye.
Mannitol Salt Agar	Yellow to orange, surrounded by yellow zones.
Staphylococcus Medium 110	Yellow to orange.
Tellurite Glycine Agar	Black.
VJ Agar	Black, surrounded by yellow zones.
Baird Parker Agar	Grey to black shiny colonies surrounded by zones of clearing.
Gram Stain:	Gram-positive cocci occurring in grape-like clusters or, occasionally, in chains.
Catalase Test:	Positive.
Mannitol Fermentation:	Positive.

3. Using a bacteriological loop, transfer a well-isolated colony from a pure culture into a tube of sterile Brain Heart Infusion broth. Incubate for 18-24 hours or until a dense growth is observed.

   Alternatively, 2-4 colonies (1 loopful) taken directly from a noninhibitory agar plate may be used as an inoculum instead of a broth culture.

Germ Tube Development

1. Obtain a pure culture of the organism to be tested. Select well-isolated colonies grown on Sabouraud Dextrose Agar for 48-72 hours.

Test Procedure

Coagulase Test

1. Using a sterile 1 ml pipette, add 0.5 ml of rehydrated Coagulase Plasma or Coagulase Plasma EDTA to a 12 x 75 mm test tube supported in a rack.
2. Using a sterile 1 ml serological pipette, add 2 drops of the overnight broth culture of the test organism to the tube of plasma or, using a sterile bacteriological loop, thoroughly emulsify 2-4 colonies (1 loopful) from a noninhibitory agar plate in the tube of plasma.
3. Mix gently.
4. Incubate in a waterbath at 35-37°C for up to 4 hours.
5. Examine the tube for coagulation hourly until a clot is evident or until 4 hours have elapsed. If no clot has formed within 4 hours, reincubate and examine after 24 hours.

   Examine by gently tipping the tube. Avoid shaking or agitating the tube, which could cause breakdown of the clot and, consequently, doubtful or false-negative test results.
6. Record results.

Germ Tube Test

1. Using a sterile 1 ml pipette, add 0.5 ml of the rehydrated Coagulase Plasma (citrated) to a 12 x 75 mm test tube in a rack.
2. Touch the tip of a sterile Pasteur pipette to a yeast colony growing on a Sabouraud Dextrose Agar plate.
3. Gently emulsify the cells in the tube of rehydrated plasma.
4. Incubate the mixture in a waterbath at 37°C for 2-4 hours.
5. Examine 1 drop of the incubated mixture microscopically for germ tubes.
6. Record results.

Results

Coagulase Test

Any degree of clotting in Coagulase Plasma or Coagulase Plasma EDTA is considered a positive test.

Germ Tube Test

The development of short, lateral hyphal filaments (germ tubes) on the individual yeast cells with no constriction at the point of attachment is considered a positive test.

Limitations of the Procedure

1. The slide agglutination technique for determining the coagulase activity of staphylococci is not recommended because false-positive reactions may occur with some strains when animal plasmas are used. In addition, spontaneous agglutination may occur when rough cultures are used. Because 10-15% of *S. aureus* isolates may yield a negative result when this test is employed, all negative slide reactions must be confirmed by the tube test.

2. Some species of organisms utilize citrate in their metabolism and will yield false-positive reactions for coagulase activity. Normally, this would not cause problems since the coagulase test is performed almost exclusively on staphylococci. However, it is possible that bacteria which utilize citrate may contaminate *Staphylococcus* cultures on which the coagulase test is being performed. These contaminated cultures may, upon prolonged incubation, give false-positive results due to citrate utilization.[3]
3. When checking results of the Coagulase Test, observe tubes hourly during the first 4 hours of incubation. Some strains of *S. aureus* produce fibrinolysin, which may lyse clots. If the tubes are not read until 24 hours of incubation, reversion to a false-negative may occur.[26]
4. Do not use plasmas if a heavy precipitate or clot has formed before inoculation.

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Packaging

Coagulase Plasma	6 x 3 ml	0286-46
	6 x 15 ml	0286-86
	6 x 25 ml	0286-66
Coagulase Plasma EDTA	6 x 3 ml	0803-46
	6 x 15 ml	0803-86
	6 x 25 ml	0803-66

Bacto® E. Coli Antisera
E. Coli O Antiserum O157 · E. Coli H Antiserum H7

Intended Use

Bacto E. Coli O Antiserum O157 and E. Coli H Antiserum H7 are used for identifying *Escherichia coli* O157:H7.

Summary and Explanation

E. coli O157:H19 was described in 1972 as a causative agent of diarrhea in swine.[1] The H19 flagellar antigen was the common antigen for serogroup O157. An H7 variant of somatic group O157 has been incriminated in severe hemorrhagic colitis.

E. coli O157:H7 is a foodborne pathogen that can cause potentially fatal enteric-related disease in humans.[2,3,4,5,6,7,8] This disease is characterized by sudden onset of severe cramps and abdominal pain, followed by a watery stool that may become markedly bloody. A series of 107 outbreaks involving 387 persons was traced to imported Camembert cheese in the United States in 1971.[9] E. coli O157:H7 was recognized as a cause of hemorrhagic colitis in 1982[5], and hemolytic uremic syndrome in 1983.[10] The 1982 outbreak was derived from ingested hamburgers.[5,11]

The incidence of disease caused by this organism has increased significantly over the past decade.[4,12] The largest outbreak of *E. coli* O157:H7 disease occurred during January 1993, in Washington State, where more than 600 patients with hemorrhagic colitis were confirmed.[2] The source of the outbreak was identified as undercooked hamburger at multiple outlets of the same fast food restaurant chain.

E. coli O157:H7 is an enteric pathogen that requires only a low inoculum to cause disease. Transmission is usually via high volume food items whose preparation is not always under stringent control and is served to a target audience (children and the elderly) most at risk for complications of illness. The organism has been isolated from several foods, including undercooked hamburger, drinking water, new potatoes, turkey roll, raw milk and apple cider. Serotyping of the entero-hemorrhagic *E. coli* is useful in the epidemiological documentation of the spread of a particular strain in a foodborne outbreak.[12]

User Quality Control

Identity Specifications

E. Coli O Antiserum O157

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

E. Coli H Antiserum H7

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Straw colored, clear solution.

Cultural Response

Rehydrate E. Coli O Antiserum O157 and E. Coli H Antiserum H7 per label directions. Test as described (see Test Procedure). Known positive and negative control cultures must give appropriate reactions.

ORGANISM	ATCC®	REACTION
E. coli O157:H7	35150	Positive
E. coli O111:K58:H21	29552	Negative

These strains may be used for Quality Control. All cultures should be serologically validated before use.

Principles of the Procedure

E. Coli O Antiserum O157 is used in the tube technique for O antigen titration. E. Coli H Antiserum H7 is used in the tube agglutination technique for detecting H antigens. These antisera are used to confirm the presence of *E. coli* O157:H7 after selective isolation. For isolation of this organism from food, use MacConkey Sorbitol Agar.[13] MacConkey Sorbitol Agar has also been used successfully to isolate *E. coli* O157:H7 from stool specimens. However, high levels of contaminating coliform organisms will mask the O157 strains.[14]

Procedures for serological confirmation by E. Coli O Antiserum O157 and E. Coli H Antiserum H7 require a pure culture of the test organism isolated on Veal Infusion Agar or other enriched solid medium. The serological technique is based on the reaction of a specific antiserum with its homologous antigen. While the specificity of serological methods is not absolute, serotyping of *E. coli*, taken with biochemical characteristics, can provide an accurate identification of the etiological agent.

Reagents

E. Coli O Antiserum O157 and E. Coli H Antiserum H7 are lyophilized, polyclonal rabbit antisera containing approximately 0.04% Thimerosal as a preservative.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated E. Coli Antisera at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

E. Coli O Antiserum O157
E. Coli H Antiserum H7

Materials Required But Not Provided

Veal Infusion Agar
Motility GI Medium

Test tubes (12 x 75 mm) or other suitable test tubes and rack
Sterile 0.85% NaCl solution
Formalin
1 ml serological pipettes
McFarland Standard No. 3
Waterbath (50 ± 2°C)

Method of Preparation

E. Coli Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to dissolve contents completely. The rehydrated antiserum is considered a 1:2 working dilution.

Tube Technique for O Antigen Titration

1. To prepare pure cultures of the test organism, plate the organism on Veal Infusion Agar and incubate at 35 ± 2°C for 16-18 hours.
2. Suspend some growth from the solid medium in 0.85% NaCl solution to give a homogeneous suspension.
3. Heat the bacterial suspension in a boiling water bath for 30-60 minutes. The culture should be homogeneous. Precipitation indicates a rough culture and the suspension should be discarded.
4. Allow the suspension to cool; dilute with 0.85% NaCl solution to a density approximating that of a McFarland Barium Sulfate Standard No. 3.
5. Add formalin to a final concentration of 0.5% by volume.
6. In a rack, prepare a row of 8 culture tubes (12 x 75 mm) for each test suspension
7. Dispense 0.9 ml of 0.85% NaCl solution in the first tube of each row and 0.5 ml in the remaining tubes.
8. **E. Coli O Antiserum O157**: Prepare serial dilutions using the rehydrated antiserum, which is already at a 1:2 working dilution. Dispense 0.1 ml of antiserum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Proceed in like manner for each suspension to be tested. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.

   This procedure yields antiserum dilutions of 1:20-1:1280.
9. **Heated bacterial suspension**: Add 0.5 ml to each of the 8 tubes. Final antiserum dilutions are 1:40-1:2560.
8. Incubate in a waterbath at 50 ± 2°C for 18-20 hours. Read for agglutination.

Tube Agglutination Technique for H Antigen Detection

1. Prepare an actively motile culture of the suspect E. coli culture by several successive transfers in Motility GI Medium. At least 2-3 passages through Motility GI Medium are necessary before attempting to establish the presence and identity of H antigens. Fresh isolates of *E. coli* generally have poorly developed flagella.
2. Inoculate a loopful of the Motility GI Medium culture into a tube of Veal Infusion Broth. Incubate 6-8 hours at 35 ± 2°C or overnight, if necessary.
3. Inactivate the culture by adding formalin to a final concentration of 0.3% (0.3 parts formaldehyde per 100 parts of the Veal Infusion Broth culture). If necessary, adjust the density of the suspension with formalinized saline to approximate a McFarland Barium Sulfate Standard No. 3. This broth culture will be used as the test antigen in step 6.
4. **E. Coli H Antiserum H7**: Prepare a 1:500 dilution by adding 0.2 ml of rehydrated antiserum, which is already a 1:2 working dilution, to 49.8 ml of 0.85% NaCl solution.
5. Pipette 0.5 ml of the antiserum dilution into a test tube.
6. **Test antigen**: Add 0.5 ml to the above dilution and shake well. The resulting antiserum dilution will be 1:1,000.
7. Incubate the tube in a 50 ± 2°C waterbath for 1 hour and read for agglutination.

Results

Observe test results with indirect lighting against a dark background. Record as follows.

- 4+ 100% agglutination of cells; supernatant fluid is clear to very slightly hazy.
- 3+ 75% agglutination of cells; supernatant fluid is slightly cloudy.
- 2+ 50% agglutination of cells; supernatant fluid is moderately cloudy.
- 1+ 25% agglutination of cells; supernatant fluid is cloudy.
- ± Less than 25% agglutination of cells.
- − No agglutination.

E. Coli O157: Cultures showing 2+ or greater agglutination at a dilution of 1:320 or greater are considered positive.

E. Coli H7: Tubes showing 2+ or greater agglutination are considered positive.

Limitations of the Procedure

1. Final identification of *E. coli* O157 is based on biochemical reactions and the presence of the O antigen.
2. The test organism must be identified to at least the genus level and, in some cases, to the species level biochemically before serotyping *E. coli*.
3. If the antiserum is cloudy after rehydration, check its bacterial purity and the pH of the saline. Discard any serum that is cloudy and/or has a precipitate unless it has been clarified and shown to react properly with known control cultures.
4. Adhere strictly to the time limitations in both tests.
5. Exposure of the organism or plate to heat from external sources (a hot bacteriological loop, burner flame, light source, etc.) may result in either a culture that cannot be suspended readily or evaporation and/or precipitation of the test mixture. These conditions may cause false-positive reactions.
6. The test culture must be checked in a saline control for smoothness. Stock cultures and, sometimes, isolated cultures may be rough and will agglutinate spontaneously in a normal serum. Therefore, it is necessary to select smooth colonies for serological testing.
7 In *E. coli* serology, as in any serological test, known positive and negative control cultures should be employed.
8. Antisera should not be subjected to repeated freezing and thawing. Such treatment is detrimental to antibody content.

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Packaging

E. Coli O Antiserum O157	3 ml	2970-47
E. Coli H Antiserum H7	3 ml	2159-47

Bacto® FA Bordetella Pertussis
Bacto FA Bordetella Parapertussis

Intended Use

Bacto FA Bordetella Pertussis and Bacto FA Bordetella Parapertussis are used for identifying *Bordetella pertussis* and *Bordetella parapertussis* by the direct fluorescent antibody technique.

User Quality Control

Identity Specifications

FA Bordetella Pertussis, FA Bordetella Parapertussis

Lyophilized appearance: Yellow button to powdered cake.

Rehydrated appearance: Yellow, clear solution.

Performance Response

Rehydrate FA Bordetella and FA Bordetella Parapertussis per label directions. Perform the fluorescent antibody staining procedure using appropriate known *Bordetella pertussis* and *Bordetella parapertussis* cultures as homologous and heterologous controls. The positive control should produce a 4+ reaction using the working dilution of the conjugate. The negative control should not exceed a 1+ reaction using the working dilution of the conjugate.

Summary and Explanation

All members of the genus *Bordetella* are respiratory pathogens of warm-blooded animals. *B. pertussis* and *B. parapertussis* are two uniquely human species. These organisms adhere to, multiply among and remain localized in the ciliated epithelial cells of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *Bordetella parapertussis* is associated with a milder, less frequently occurring form of the disease.[1] Person-to-person transmission occurs by the aerosol route.

Pertussis is a highly contagious disease that attacks unimmunized populations in more than 90% of cases.[2] Toxin production remains the major distinction of *B. pertussis*.

Classic pertussis caused by *B. pertussis* occurs in three stages. The first or catarrhal stage is characterized by nonspecific symptoms similar to a cold or viral infection. The disease is highly communicable during this stage, which lasts 1-2 weeks. In the second or paroxysmal stage, the cough increases in intensity and frequency. This stage is marked by sudden attacks of severe, repetitive coughing, often cumulating with the characteristic whoop that is caused by a rapid inspiration of air after the clearance of mucus-blocked airways.[3] This stage may last 1-4 weeks. The beginning of the convalescent stage is marked by a reduction in the frequency and severity of coughing spells. Complete recovery may require weeks or months.

Despite the availability of an effective whole-cell vaccine, pertussis remains a disease of worldwide distribution because many developing nations do not have the resources for vaccinating their populations.[4] Major outbreaks have occurred even in developed nations such as Great Britain and Sweden. Pertussis is endemic in the United States, with most disease occurring as isolated cases. A shift in the age group affected by the disease has occurred. In the past, children in the 1-5 year age group were more prone to pertussis. Children less than one year of age[2] have become more susceptible to the disease because of a decrease in passively transferred maternal antibodies, since adults do not receive booster vaccinations.

Bordetella are tiny gram-negative coccobacilli occurring singly or in pairs and they may exhibit a bipolar appearance. They are strict aerobes and some members are motile. *B. pertussis* and *B. parapertussis* are nonmotile and produce no acid from carbohydrates. *B. pertussis* will not grow on common blood agar bases or chocolate agar, whereas *B. parapertussis* will grow on blood agar and sometimes chocolate agar. Media for primary isolation must include starch, charcoal, ion-exchange resins or a high percentage of blood to inactivate inhibitory substances.[3] *B. pertussis* may be recovered from secretions collected from the posterior nasopharynx, bronchoalveolar lavage and transbronchial specimens.

The direct fluorescent antibody test (DFA) has long been used for the rapid, direct detection of *B. pertussis* and *B. parapertussis* in nasopharyngeal specimens with varying degrees of success.[5,6,7] Eldering, Eveland and Kendrick[8,9] and Holwerda and Eldering[10] showed the usefulness of the FA procedure, although a complete correlation between the agglutination method and FA technique was not obtained. Nonetheless, the FA procedure could detect both smooth and rough cultures of *B. pertussis* and *B. parapertussis* and could also be applied to direct specimens. Further data showed little or no cross reactions between conjugates prepared from *B. pertussis* and *B. parapertussis* cultures. The disadvantage of this procedure is that technical skill and experience are required for the technician to perform and read the test. The DFA should always be used with, not as a replacement for, culture.[11,12] It has been suggested that laboratories proficient in DFA and culture for pertussis should obtain a DFA sensitivity of 60% or greater and a specificity of at least 90% over time compared with culture.[12]

B. pertussis and *B. parapertussis* are slow growing organisms, developing in 3-4 days.

By employing the fluorescent antibody technique, the time required to detect these organisms can be significantly reduced. The FA procedure may be applied to direct nasopharyngeal smears or may be used to identify young cultures of *B. pertussis* or *B. parapertussis.*

Principles of The Procedure

The direct FA technique involves preparation of a smear from the clinical specimen on a glass slide. Nasopharyngeal swabs are obtained from the patient and inoculated into 0.5 ml of Casamino Acids solution.[12] Smears are made from this solution and stained with a specific antibody labeled with a fluorescent marker (fluorescein isothiocyanate or FITC) directed against *B. pertussis* or *B. parapertussis*. After incubation with the antibody preparation, smears are washed in phosphate-buffered saline, air dried, cover slipped with fluorescent-antibody mounting fluid, and examined under a fluorescent microscope.

Reagents

FA Bordetella Pertussis and **FA Bordetella Parapertussis** are lyophilized, polyclonal, fluorescein-conjugated chicken antisera. They have been prepared according to modifications of the methods of Eldering, Eveland and Kendrick[8,9] and Holwerda and Eldering.[1] Approximately 0.02% Thimerosal is added as a preservative.

Precautions

1. For In Vitro Diagnostic Use.
2. **FA Bordetella Pertussis**
   FA Bordetella Parapertussis
   The Packaging of This Product Contains Dry Natural Rubber.
3. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[13,14]
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized FA Bordetella Pertussis and FA Bordetella Parapertussis at 2-8°C.

Aliquots of the titered conjugate may be prepared in small vials, frozen in the undiluted state and stored below -20°C for optimal stability. The conjugate should not be exposed to repeated freezing and thawing.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

FA Bordetella Pertussis
FA Bordetella Parapertussis

Materials Required But Not Provided

FA Buffer, Dried
FA Mounting Fluid pH 7.2
1% Casamino Acids
Staining Tray
Fluorescent microscope assembly:

Lamps:	HBO-50, HBO-100, HBO-200 or Xenon XBO-150; 6X 5A Tungsten
Excitation wavelength:	365 nm
Ocular:	10X
Objective:	10X, 40X (Fluorite)
Filters:	BG-12 or KP490, K515 or K530
Condenser:	Dark-field D1.20-1.40

Microscope slides
95% ethanol
Staining jar
Cover slips
McFarland Barium Sulfate Standard #3

Reagent Preparation

Equilibrate all materials to room temperature before performing the test. Ensure that all glassware and pipettes are clean and free of detergent residues.

FA Bordetella Pertussis and FA Bordetella Parapertussis: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

The working dilution of the conjugate should be determined shortly after rehydration. The titer of a conjugate varies with the technique used, the fluorescent microscope and filter used, and the age of the bulb.

The conjugate should be titrated using a known culture of *B. pertussis* or *B. parapertussis* homologous to the conjugate. (Dilutions of the conjugate are made in FA Buffer.) The titer is determined as follows:

DILUTION OF CONJUGATE	FLUORESCENCE
1:5	4+
1:10	4+
1:20	4+
1:40	4+
1:80	2+

In this example, the last 4+ fluorescence is found in a 1:40 dilution of the conjugate. One less dilution is chosen for a margin of safety. The working dilution in this case is, therefore, 1:20.

Specimen Collection and Preparation

Direct Nasopharyngeal Smears

1. Obtain a nasopharyngeal swab and emulsify it in 0.5 ml of sterile 1% Casamino Acids.[12]
2. Hold the specimen in Casamino Acids solution for no more than 2 hours.
3. Smear the emulsified specimen on a clean microscope slide.
4. Allow the smear to air dry and fix it by gentle heating or by a 1 minute immersion in 95% ethanol.

Culture Isolates

1. Isolation of *Bordetella* from clinical specimens requires the use of certain media such as Bordet-Gengou Agar. Colonies of *B. pertussis* on Bordet-Gengou Agar or Charcoal Agar are very small, white, opaque, convex and entire. For specific recommendations, consult appropriate references.[3,12] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as a *Bordetella* species. After these criteria are met, serological identification can be performed.
2. Pick appropriate colonies and emulsify in approximately 2 ml sterile distilled or deionized water. Adjust cell density to approximate a McFarland Barium Sulfate Standard #3.
3. Smear the emulsified specimen on a clean microscope slide.
4. Allow the smear to air dry and fix it by a 1 minute immersion in 95% ethanol. Remove slides and allow them to air dry.

Control Slides

Prepare positive and negative control slides using appropriate homologous antigens, following the procedure listed under Culture Isolates.

Test Procedure

1. Add several drops (one drop equals ~35 µl) of the appropriate FA Bordetella conjugate to the fixed smear.
2. Spread the conjugate over the surface of the smear.
3. Place the slide in a Staining Tray or moisture chamber.
4. Incubate at room temperature for 30 minutes.
5. Remove excess conjugate and place the slide in a staining jar containing FA Buffer for 10 minutes with 2 changes of the buffer followed by 1 rinse in distilled water for 2 minutes.
6. Remove the slide; allow to drain and air dry or blot with bibulous paper.
7. Add a small drop (~35 µl) of FA Mounting Fluid pH 7.2 to the center of the stained area and mount with a cover slip.
8. Examine each smear using a fluorescent microscope with an excitation wavelength of 365 nm under a 40X or 100X objective. Record the absence or presence and degree of fluorescence.

Results

1. Read and record results based on the intensity of fluorescence, as follows:
   - 4+ Maximum fluorescence; brilliant yellow-green peripheral staining.
   - 3+ Bright yellow-green peripheral staining.
   - 2+ Definite, but dull, yellow-green peripheral staining.
   - 1+ Barely visible peripheral staining.
   - – Complete absence of yellow-green peripheral fluorescence.
2. **Positive control**: Should show a 4+ reaction using the working dilution of the conjugate.
   Negative control: Should not exceed a 1+ reaction using the working dilution of the conjugate.
   Test smears: A 2+ fluorescence should be considered a positive result.
3. If the positive control is less than 3+, or if the negative control exceeds 1+, the conjugate may have deteriorated or the pH of the FA Buffer or FA Mounting Fluid may have changed. Repeat the test with new reagents.

Limitations of the Procedure

1. At the Routine Test Dilution (RTD) of both FA Bordetella Pertussis and FA Bordetella Parapertussis, the reaction should be brilliant and specific with smooth strains of homologous cultures. Usually, there are no cross reactions with the heterologous strains at the RTD. With some heterologous strains, a 1+ reaction may occur. Such a minimal reaction should not interfere with the interpretation of the test results.

   Some strains of *Bordetella bronchiseptica*, on the other hand, may cross react with both conjugates in varying degrees, giving 1+ to 4+ reactions with a small population of the cells in the smear. The majority of the cell population, however, should not stain at more than a 2+ intensity.
2. Some experience is required to grade the intensity of the fluorescence and to ignore the occasional nonspecific staining of gram-negative diplococci, gram-positive cocci and diphtheroid-like rods.[12]
3. When testing cultural isolates, the density of the positive control should be adjusted to give 4+ fluorescence with the homologous conjugate. The density of culture isolates should be comparable to the positive control in order to standardize fluorescence.

4. All glassware employed in the preparation, testing and storage of these reagents must be free of detergents or other harmful residues.
5. The fluorescent antibody technique can provide only presumptive identification of *B. pertussis* or *B. parapertussis*. A negative result should not be considered conclusive as this type of reaction may occur when only a few organisms are present in the specimen. Final identification can be made only after consideration of cultural, morphological and serological characteristics.

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3. **Marcon, M. J.** 1995. *Bordetella*, p. 566-573. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
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13. **Centers for Disease Control.** 1988. Update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. Morbidity and Mortality Weekly Reports **37:**377-382, 387-388.
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Packaging

FA Bordetella Pertussis	5 ml	2359-56
FA Bordetella Parapertussis	5 ml	2378-56

Bacto® FA Product Accessories and Reagents

FA Buffer, Dried · FA Mounting Fluid pH 7.2 · FA Mounting Fluid pH 9 · Staining Tray

Intended Use

Bacto FA Buffer, Dried is used in fluorescent antibody (FA) staining procedures.

Bacto FA Mounting Fluid pH 7.2 is used in FA procedures to mount specimens on slides at pH 7.2.

Bacto FA Mounting Fluid pH 9 is used in FA procedures to mount specimens on slides at pH 9.

The Staining Tray is used in FA staining procedures.

Summary and Explanation

FA Buffer, Dried is a phosphate buffer-NaCl mixture which, upon rehydration, yields a 0.85% NaCl solution buffered to pH 7.2. It is used for making dilutions of rehydrated FA Globulins for test purposes, as well as for washing slides in FA staining procedures. It is also recommended as a general purpose phosphate buffered saline.

FA Mounting Fluids are buffered glycerine preparations used in fluorescent antibody procedures as a semipermanent mounting medium for specimens on slides. With mounting media, the cover slip should be pressed down firmly to reduce hazy images. Add a very small drop of mounting fluid and avoid forming bubbles.

Mounting media may be adjusted to any pH compatible with the fluorescence of the fluorochrome to be used. For FITC procedures, the pH should not be lower than 7.0 because fluorescence decreases rapidly below this pH. FITC preparations may be mounted at pH of 9.0 to increase the intensity of fluorescence;[1] however, nonspecific staining is also increased.[2] The pH of a mounting fluid decreases with

time because of oxidation of the glycerol and absorption of CO_2 by the mounting fluid.[3]

The Staining Tray provides a moist, dark incubation chamber for FA conjugated slides.

Principles of the Procedure

Slides are stained using fluorescent antibody procedures. After removal of excess conjugate or serum, a drop of the appropriate FA Mounting Fluid pH 7.2 or FA Mounting Fluid pH 9 is added. A cover slip is applied, taking care not to form bubbles when the cover slip is added.

Reagents

FA Buffer, Dried is a phosphate buffer-NaCl mixture which, upon rehydration, yields a 0.85% NaCl solution buffered to pH 7.2.

FA Mounting Fluid pH 7.2 and FA Mounting Fluid pH 9 are standardized, reagent grade glycerin adjusted to pH 7.2 and 9.0, respectively.

Precautions

1. For In Vitro Diagnostic Use.
2. **FA Buffer, Dried**

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

FA Buffer, Dried

Dehydrated Appearance:	White, free flowing, homogeneous.
Solution:	solution, soluble in distilled or deionized water. Solution is colorless, clear.
Reaction of 1% Solution at 25°C:	pH 7.2 ± 0.05

FA Mounting Fluid pH 7.2

Appearance:	Colorless, clear, free from lint.
pH at 25°C:	7.2 ± 0.1

FA Mounting Fluid pH 9

Appearance:	Colorless, clear, free from lint.
pH at 25°C:	9.0 ± 0.1

Performance Response

Perform the fluorescent antibody procedure using an appropriately titrated conjugate. Rinse off excess conjugate. Add a cover slip with an appropriate FA Mounting Fluid. Read smears with a fluorescent microscope. The FA Mounting Fluid pH 7.2 or FA Mounting Fluid pH 9 must not show “quenching” of fluorescence and must show 4+ fluorescence for all slides tested with the homologous antigen.

Storage

Store dehydrated FA Buffer, Dried below 30°C. Upon rehydration, store FA Buffer, Dried at 2-8°C.

Store FA Mounting Fluid pH 7.2 and FA Mounting Fluid pH 9 at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

FA Buffer, Dried

Refer to appropriate procedures for FA Bordetella, Fluorescent Treponemal Antibody Testing (FTA-ABS), FA Streptococcus Group A or FA Rhodamine Counterstain.

FA Mounting Fluid pH 7.2

Refer to appropriate procedures for FA Bordetella or FTA-ABS.

FA Mounting Fluid pH 9

Refer to appropriate procedures for FA Streptococcus Group A or FA Rhodamine Counterstain.

Limitations of the Procedure

1. An acid pH will cause a marked decrease in fluorescence.
2. Fresh mounting fluid should be used. The fluid should be discarded if any color or turbidity appears.[2]

References

1. **Pital, A., and S. L. Janowitz.** 1963. Enhancement of staining intensity in the fluorescent-antibody reaction. J. Bacteriol. **86:**888-889.
2. **Cherry, W. B.** 1974. Immunofluorescence techniques, p. 29-44. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
3. **Anhalt, J. P.** 1981. Fluorescent antibody procedures and counterimmuno-electrophoresis, p. 249-277. *In* J. Washington (ed.), Laboratory procedures in clinical microbiology. Springer-Verlag Inc, New York, NY.

Packaging

FA Buffer, Dried	6x10 ml	2314-33
	100 g	2314-15
	10 kg	2314-08
FA Mounting Fluid pH 7.2	6x5 ml	2329-57
FA Mounting Fluid pH 9	6x5 ml	3340-57
Staining Tray	1 tray	5251-31

Bacto® FA Streptococcus Group A

Intended Use

Bacto FA Streptococcus Group A is used for identifying Group A Streptococcus by the direct fluorescent antibody technique.

Summary and Explanation

Streptococcus pyogenes (Group A streptococcus) is the most common cause of bacterial pharyngitis in children. Symptoms include fever, pharyngeal erythema and edema, tonsillar exudate and enlarged cervical lymph nodes. Physical findings alone cannot distinguish between Group A streptococcal pharyngitis and pharyngitis caused by other agents such as viruses or mycoplasma. Other infections caused by Group A streptococci include scarlet fever, impetigo and skin infections that range from mild to severe with toxic shock symptoms and tissue necrosis.

Streptococci are facultatively anaerobic gram-positive cocci. They are catalase negative and may be alpha, beta or non-hemolytic. Lancefield divided the streptococci into serological groups according to the group-specific somatic carbohydrate they possessed.[1,2,3] The Lancefield groups have quite different clinical significance. There may be biochemical and hemolytic differences within the same serological group.

Moody, Ellis and Updyke[4] showed that group-specific conjugates could be prepared from the antiserum used in the Lancefield precipitin test. This led to the development of the direct fluorescent antibody technique for the identification of *Streptococcus* groups. The Group A conjugate, prepared according to the method of Moody, Ellis and Updyke[4] and Moody, Siegel, Pittman and Winter[5], is used for the detection and identification of group A *Streptococcus*.[6-8]

Principles of the Procedure

The direct FA technique involves the preparation of a smear from the clinical specimen on a glass slide. Smears are ethanol-fixed and stained with a specific antibody labeled with a fluorescent marker (fluorescein isothiocyanate or FITC) directed against Group A *Streptococcus*. The antigen-antibody reaction is then observed microscopically, using a suitable wave length of light compatible with the fluorescent marker employed.

User Quality Control

Identity Specifications

FA Streptococcus Group A

Lyophilized Appearance: Yellow button to powdered cake.

Rehydrated Appearance: Yellow, clear solution.

Performance Response

Rehydrate FA Streptococcus Group A per label directions. Perform the fluorescent antibody staining procedure using a known culture of Group A *Streptococcus* as the homologous control. The positive control should produce a 4+ reaction in the 1:20 or greater dilution of the conjugate with the homologous antigen.

Reagents

FA Streptococcus Group A conjugate has been cross-absorbed to remove cross reactivity known to exist with serogroups C and G. Normally occurring *Staphylococcus* agglutinins have been blocked by unconjugated normal rabbit serum or by an unconjugated *Staphylococcus* immune serum. Approximately 0.02% Thimerosal is used as a preservative.

The working dilution of the conjugate should be determined upon rehydration. The titer of a conjugate varies with the technique, the fluorescent microscope, the filter and the age of the bulb. The working dilution may vary from laboratory to laboratory. Dilutions of the conjugate are made in rehydrated FA Buffer. The titer is determined as follows:

DILUTION OF CONJUGATE	FLUORESCENCE
1:5	4+*
1:10	4+
1:20	4+
1:40	4+
1:80	2+

*4+ fluorescence is defined as brilliant yellow-green cocci with sharp cell outlines and nonstaining centers.

In this example, the last 4+ fluorescence is found in a 1:40 dilution of the conjugate. Use one dilution lower for a margin of safety. The working dilution, therefore, in this case is 1:20.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store lyophilized FA Streptococcus Group A at 2-8°C.

Aliquots of the titered conjugate should be prepared in small vials, frozen in the undiluted state and stored below -20°C for optimal stability. Prepare only a sufficient amount of diluted conjugate for each day's use.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

FA Streptococcus Group A

Materials Required but not Provided

FA Buffer, Dried

FA Mounting Fluid pH 9

Staining Tray
Fluorescent microscope assembly:

Lamps:	HBO-50, HBO-100, HBO-200 or Xenon XBO-150; 6X 5A Tungsten
Excitation Wavelength:	365 nm
Ocular:	10X
Objective:	10X, 40X (Fluorite)
Filters:	BG-12 or KP490, K515 or K530
Condenser:	Dark-field D1.20-1.40

95% Ethanol
McFarland Barium Sulfate Standard #3
Glass slides
Cover slips
Sterile swabs
Culture tubes, 12 x 75 mm
Coplin jars
Serological pipettes, 1 ml and 5 ml
Todd Hewitt Broth
Incubator, 35 ± 2°C

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergent.

FA Streptococcus Group A: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Specimens submitted to the laboratory for the detection of streptococci must be obtained under proper medical guidance. Suitable specimens are those collected from the nose, nasopharyngeal area and throat, skin, wounds, pus, blood, cerebrospinal fluid and urine. When collecting a throat specimen, it is imperative that the sample is collected properly to obtain an adequate amount of material. Improperly obtained specimens will yield cultures that contain minimal numbers of streptococci. For specific information on specimen collection and preparation, consult appropriate references.[9,10]

Note: Specimens containing streptococci survive well at 4EC on tightly capped blood agar slants but survive poorly in a broth medium.

1. Place a swab having a throat or other specimen, excluding urine, into a tube containing 1 ml Todd Hewitt Broth and incubate at 35 ± 2°C for 2-5 hours. For urine samples, proceed to step #3.
2. Drain the swab against the side of the tube and place it into another sterile tube for storage in a refrigerator, if desired.
3. Centrifuge tubes containing known and unknown cultures for 10 minutes at 1,500-2,000 rpm to sediment cells.
4. Pour off supernatant liquid (autoclave before discarding) and resuspend the cells in 2 ml distilled or deionized water. Adjust density to approximately a McFarland Barium Sulfate Standard #3.
5. Prepare duplicate smears of the same culture on prepared microscope slides.
6. Allow the smear to air dry.
7. Fix the smear by placing the slide in 95% ethanol for 1 minute.
8. Remove the slide and allow to air dry.

Note: Smears may also be prepared from specimens grown on blood agar and suspected of being *Streptococcus* because of their growth characteristics. The isolation medium recommended for this species is any one of several blood agar bases containing 5% sterile, defibrinated sheep blood. Hemolytic reactions should be determined from a pure culture before serological examination. Sheep blood plates are recommended because they exhibit clear-cut reactions for streptococci. For antigen preparation, Todd Hewitt Broth is recommended.

Test Procedure

1. Add several drops of a predetermined working dilution of FA Streptococcus Group A conjugate to the smear on one end of a microscope slide. Distribute it evenly over the entire smear with an applicator stick so as not to disturb the smear.
2. Place the slide in a Staining Tray or moist chamber. Incubate at room temperature for 30 minutes.
3. Drain off excess conjugate and place in a Coplin jar containing FA Buffer solution. Let stand for 10 minutes with 2 changes of buffer and a final rinse of distilled water.
4. Remove the slide; allow it to drain and air dry.
5. Add one small drop of FA Mounting Fluid pH 9 to the slide and mount with a glass cover slip.
6. Examine using a fluorescent microscope with an excitation wavelength of 365 nm under a 40X or 100X objective.
7. Read and record the amount of fluorescence.

Results

1. Read and record results as follows:
   - 4+ Maximum fluorescence; brilliant yellow-green; clear-cut cell outline; sharply defined cell center.
   - 3+ 75% fluorescence; less brilliant yellow-green; clear-cut cell outline; sharply defined cell center.
   - 2+ 50% fluorescence; definite but dim; cell outline less well defined.
   - 1+ 25% agglutination; background is cloudy.
   - – Negligible or complete lack of fluorescence (negative).
2. A 4+ fluorescence in the unknown smear with the homologous conjugate is evidence that the unknown organism is homologous to Streptococcus Group A conjugate.

Limitations of the Procedure

1. Some experience is required to grade the intensity of the fluorescence.
2. Discard the conjugate if contaminated.
3. The conjugate should not be subjected to repeated freezing and thawing. Such treatment is detrimental to the antibody content.
4. All glassware employed in the preparation, testing and storage of these reagents must be free of detergents or other harmful residues.
5. FA Buffer showing turbidity or mold growth should be discarded.

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Packaging

FA Streptococcus Group A	5 ml	2318-56
FA Buffer, Dried	6 x 10 ml	2314-33
	100 g	2314-15
	10 kg	2314-08
FA Mounting Fluid pH 9	6 x 5 ml	3340-57
Staining Tray	1 tray	5251-31

Bacto® FTA-ABS Test Reagents

FTA Serum Reactive · FTA Antigen · FTA Serum Non-Reactive FTA Sorbent · FTA Sorbent Control · FA Human Globulin Antiglobulin (Rabbit) · Tween® 80 · FA Buffer, Dried FA Mounting Fluid pH 7.2

Intended Use

The FTA-ABS Test (Fluorescent Treponemal Antibody Absorption) is an indirect immunofluorescent procedure for detecting human antibody against *Treponema pallidum*, the causative agent of syphilis. The test uses the following reagents: FTA Antigen, FTA Serum Reactive, FTA Serum Non-Reactive, FTA Sorbent, FTA Sorbent Control, FA Human Globulin Antiglobulin (Rabbit), Tween® 80, FA Buffer, Dried and FA Mounting Fluid pH 7.2.

The persistent reactivity of the FTA-ABS Test to a treated case of syphilis, sometimes for life, minimizes its use for following the response to therapy as well as making it unreliable for detecting new untreated cases in epidemiological investigations.

Bacto FTA Reagents are not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.[12]

Summary and Explanation

Treponema pallidum is the causative agent of syphilis, a chronic infection with many clinical manifestations. These manifestations occur in distinct stages and detection of each stage requires different laboratory tests.

During the primary stage, treponemes present in the characteristic lesion, a chancre, are detectable by dark-field microscopy[1] or by the Direct Fluorescent Antibody Test for *Treponema pallidum* (DFA-TP). During the secondary stage, most serology tests for syphilis are reactive and treponemes may be found in the lesions by using dark-field microscopy. The latent period, which is asymptomatic, may last for years. Serological tests are usually reactive in the early latent period but reactivity in non-treponemal tests decreases during the late latent period. Symptoms of the tertiary or late stage of syphilis may occur 10-20 years after initial infection. Approximately 71% of patients in the tertiary stage of syphilis have reactive non-treponemal tests.[2,3] In the tertiary stage, treponemal tests will usually be reactive and are the only basis for diagnosis. The lesions in tertiary syphilis will have few treponemes. Neurosyphilis is a complication of tertiary syphilis.

Since the clinical manifestations of syphilis can be confused with other infectious diseases or with noninfectious conditions that cause skin lesions, proper diagnosis must be based on microscopic examination of lesion material and serological test results.[2]

The FTA test was introduced in 1957 by Deacon, Falcone and Harris.[4] Certain difficulties were encountered with respect to sensitivity versus specificity. In its original form using a 1:5 dilution of patient serum, the test yielded many false-positive reactions. There seemed to be a cross reaction of the treponemal antigen with antibodies to group antigens that are common to all treponemes. The titer of sera containing the nonspecific group antibodies ranged from 1:5 to 1:100.

In 1960, Deacon, Freeman and Harris[5] introduced a modified procedure, the FTA-200 test, which used a 1:200 dilution of patient serum. By increasing the dilution of the serum, nonspecific antibodies were

diluted beyond their titer and could no longer interfere with the test. However, testing a highly diluted serum decreased the sensitivity of the test. Low antibody titer, which occurs during primary syphilis, was not detected.

Deacon and Hunter[6] showed that appropriate absorption could eliminate or block the reactivity of nonspecific antibodies. This absorption produced the FTA-ABS test, an improved test using a 1:5 serum dilution.[7]

The FTA-ABS test is a standard diagnostic test for syphilis as defined by the Centers for Disease Control and Prevention (CDC). Other standard treponemal tests include Fluorescent Treponemal Antibody-Absorption Double Staining Test (FTA-ABS DS) and the Micro Hemagglutination Assay for Antibodies to *Treponema pallidum* (MHA-TP).

Treponemal antigen tests, such as the FTA-ABS test, are used as confirmatory tests in diagnostic problem cases, such as with patients for whom the clinical, historical or epidemiological evidence of syphilis disagrees with nontreponemal tests. The FTA-ABS test is more sensitive than the VDRL test in primary, late latent and tertiary syphilis. However, the persistent reactivity of the FTA-ABS test to a treated case of syphilis, sometimes for life, minimizes its use for following response to therapy. Therefore, the FTA-ABS test is also unreliable in detecting new untreated cases in epidemiological investigations. The test should not be used as a routine screening procedure.[3,8]

The likelihood of obtaining a reactive FTA-ABS test result in various stages of untreated syphilis has been reported as follows:[2]

STAGE OF UNTREATED SYPHILIS	% REACTIVE
Primary	84
Secondary	100
Latent	100
Tertiary (Late)	96

User Quality Control

Identity Specifications

FTA Antigen
Lyophilized Appearance: White button to powdered cake.
Rehydrated Appearance: White to off-white, slightly opalescent liquid.

FTA Serum Reactive
Lyophilized Appearance: Off-white to light amber, button to powdered cake.
Rehydrated Appearance: Light gold to slightly amber liquid.

FTA Serum Non-Reactive
Lyophilized Appearance: Off-white to light amber, button to powdered cake.
Rehydrated Appearance: Light gold to slightly amber liquid.

FTA Sorbent
Lyophilized Appearance: Light amber to dark brown, button to powdered cake.
Rehydrated Appearance: Gold to brown liquid.

FTA Sorbent Control:
Lyophilized Appearance: Off-white to light amber, button to powdered cake.
Rehydrated Appearance: Light gold to slightly amber liquid.

FTA Human Globulin Antiglobulin (Rabbit)
Lyophilized Appearance: Light yellow to yellow-orange, button to powdered cake.
Rehydrated Appearance: Yellow-green to yellow-orange liquid.

Control Pattern

Rehydrate and dilute reagents per directions (see Reagent Preparation). Test as described. Tests failing to exhibit the following control results are unsatisfactory and should not be reported.[8,13]

SERUM TESTED	EXPECTED FLUORESCENCE	INTERPRETATION
Reactive Control Serum - Unabsorbed	4+	Reactive
Reactive Control Serum - Absorbed	3+ to 4+	Reactive
Minimally Reactive Control Serum	1+	Reactive
Nonreactive Control Serum	N	Nonreactive
Nonspecific Serum Control - Unabsorbed	2+ to 4+	Reactive
Nonspecific Serum Control - Absorbed	N to ±	Nonreactive
Nonspecific Staining Control - Unabsorbed	N	Nonreactive
Nonspecific Staining Control - Absorbed	N	Nonreactive

Principles of the Procedure

Patient serum is diluted 1:5 in sorbent and layered on a microscope slide fixed with *T. pallidum*. If the patient's serum contains antibodies, these antibodies will coat the treponemes on the slide. Fluorescein-labeled anti-human immunoglobulin is added. It combines with the patient antibodies already adhering to the *T. pallidum* and produces fluorescein-stained spirochetes that can be observed with a fluorescent microscope.[7,9]

Reagents

FTA Antigen (also known as *T. pallidum* antigen) is a lyophilized, standardized, killed suspension of *Treponema pallidum* (Nichols strain).

FTA Serum Reactive is lyophilized, standardized syphilitic human sera containing 0.02% Thimerosal as a preservative. It is used to make Reactive Control Serum (4+) - Unabsorbed, Reactive Control Serum (4+) - Absorbed, and Minimally Reactive Control Serum (1+). It is used as a positive control in the FTA-ABS test.

FTA Serum Non-Reactive is lyophilized, standardized, non-syphilitic human sera containing 0.02% Thimerosal as a preservative. It is used to make Nonreactive Control Serum (N). It is used as a negative control in the FTA-ABS test.

FTA Sorbent is a lyophilized, standardized extract of the nonpathogenic Reiters treponeme (*T. phagedenis*) prepared from broth culture. It is used to remove antibodies against nonpathogenic treponemes during preparation of the test specimen, Reactive Control Serum (4+) - Absorbed and Nonspecific Staining Control - Absorbed.

FTA Sorbent Control is lyophilized, standardized, non-syphilitic human sera containing 0.02% Thimerosal as a preservative. It is used to make Nonspecific Control Serum - Unabsorbed, which demonstrates at least 2+ nonspecific reactivity at a 1:5 dilution in FA Buffer, and Nonspecific Control Serum - Absorbed, which demonstrates essentially no reactivity at a 1:5 dilution in FTA Sorbent.

FA Human Globulin Antiglobulin (Rabbit) is lyophilized, fluorescein-conjugated (FITC) antihuman globulin containing 0.02% Thimerosal as a preservative. It is used to show the presence of human syphilitic antibodies on the treponemal antigen.

Tween® 80 is Polysorbate 80, U.S.P. It is used to prepare 2% Tween 80, which acts as a dispersing agent.

FA Buffer, Dried is phosphate buffered saline (PBS) which, upon rehydration, yields a 0.85% NaCl solution buffered to pH 7.2. FA Buffer is used in preparing Reactive Control Serum (4+) - Unabsorbed, Minimally Reactive Control Serum (1+), Nonreactive Control Serum (N) and Nonspecific Staining Control - Unabsorbed.

FA Mounting Fluid pH 7.2 is standardized, reagent grade glycerin adjusted to pH 7.2 for use in mounting specimens on slides to be viewed under the fluorescent microscope.

Precautions

1. For In Vitro Diagnostic Use.
2. **FTA Serum Reactive**
   FTA Serum Non-Reactive
   FTA Sorbent Control
   WARNING! POTENTIAL BIOHAZARDOUS REAGENTS. Each donor unit used in the preparation of these reagents was tested by an FDA-approved method for the presence of the antibody to human immunodeficiency virus (HIV) as well as for hepatitis B surface antigen and found to be negative (were not repeatedly reactive).

   Because no test method can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen.[10,11]
3. **FTA Antigen**
   FTA Serum Reactive
   FTA Serum Non-Reactive
   FTA Sorbent
   FTA Sorbent Control
   FA Human Globulin Antiglobulin (Rabbit)
   The Packaging of This Product Contains Dry Natural Rubber.
4. **FA Buffer, Dried**
   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
5. Observe universal blood and body fluid precautions in handling and disposing of specimens.
6. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store unopened products as specified below:

FTA Antigen	2-8°C
FTA Serum Reactive	2-8°C
FTA Serum Non-Reactive	2-8°C
FTA Sorbent	2-8°C
FTA Sorbent Control	2-8°C
FA Human Globulin Antiglobulin (Rabbit)	2-8°C in the dark
Tween® 80	15-30°C
FA Buffer, Dried	Below 30°C
FA Mounting Fluid pH 7.2	15-30°C

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Rehydrated FTA Antigen stored at 2-8°C is stable for 1 week.

Rehydrated FA Buffer showing turbidity or mold growth should be discarded.

Discard 2% Tween 80 that exhibits a precipitate or pH change.

Procedure

Materials Provided

FTA Antigen
FTA Serum Reactive
FTA Serum Non-Reactive
FTA Sorbent
FTA Sorbent Control
FA Human Globulin Antiglobulin (Rabbit)
Tween® 80
FA Buffer, Dried
FA Mounting Fluid, pH 7.2

Materials Required But Not Provided

Timer
Serological pipettes, 0.2 ml, 5 ml, 1 ml
Micropipettors delivering 10-200 µl
Test tubes, 12 x 75 mm
Water bath (56°C)
Vortex mixer
Platinum loop, 2 mm, 26 gauge
Slides, plain or frosted, 1 x 3 inch, 1 mm thick, inscribed with 2 x 1 cm circles
Staining dish with removable slide carriers
Slide board or holder
Moisture chamber
Acetone
Bibulous paper
Distilled water
Incubator, 35-37°C
Oil, Immersion
Cover slips, No. 1, 22 mm square
Fluorescent microscope assembly:

Lamps:	HBO-50, HBO-100, HBO-200 or Xenon XBO-150; 6X 5A Tungsten
Ocular:	10X
Objective:	10X, 40X (Fluorite)
Filters:	BG-12 or KP490, K515 or K530
Condenser:	Dark-field D1.20-1.40

Reagent Preparation

FTA Antigen: Rehydrate with 1 ml distilled or deionized water and rotate to completely dissolve the contents. This solution will yield

approximately 3.5 x 10^7 treponemes per ml. Mix thoroughly with a disposable pipette and rubber bulb, drawing the suspension into and expelling it from the pipette 8-10 times to break treponemal clumps and ensure an even distribution of treponemes. Confirm the even distribution by dark-field examination. Use FTA Antigen in its entirety to prepare antigen smears on the day it is rehydrated. Approximately 200-300 slides may be prepared with 1 ml of antigen.

To prepare FTA Antigen smears:

1. Wipe inscribed slides with clean gauze and, if necessary, alcohol to remove dust particles.
2. Using a platinum wire loop (2 mm, 26 gauge), smear 1 loopful of reconstituted FTA Antigen within the 2 circles. Air dry at room temperature for at least 15 minutes.
3. Immerse the dry slide into acetone for 10 minutes to fix the treponemal antigen smear to the slide; air dry. Fix no more than 50 slides per 200 ml of acetone.
4. Use slides immediately or store at or below -20°C after acetone fixation. Thaw before use; do not refreeze. Use within 1 year, but only if satisfactory results are obtained with test controls.

FTA Serum Reactive: Rehydrate with 5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Aliquot in 0.4 ml amounts and store at or below -20°C. Do not refreeze thawed aliquot. Approximately 12 tests may be obtained per 5 ml vial. This serum should be heated at 56°C for 30 minutes before use.

FTA Serum Non-Reactive: Rehydrate with 5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Aliquot in 0.4 ml amounts and store at or below -20°C. Approximately 90-100 tests may be obtained per 5 ml vial. This serum should be heated at 56°C for 30 minutes before use.

FTA Sorbent: Rehydrate with 5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Store at 2-8°C or aliquot and store at -20°C. The quantity of FTA Sorbent used for each test sample or serum is 0.2 ml. The quantity of FTA Sorbent needed for 3 controls is 0.6 ml. Approximately 20-25 tests may be performed with 5 ml of FTA Sorbent.

FTA Sorbent Control: Rehydrate with 0.5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Aliquot in 0.25 ml amounts and store at or below -20°C. For each test, 0.1 ml of FTA Sorbent Control is needed. Approximately 2 tests may be performed per 0.5 ml vial because of evaporation from heating. This serum should be heated at 56°C for 30 minutes before using.

FA Human Globulin Antiglobulin (Rabbit): Rehydrate with 1 ml or 5 ml distilled or deionized water, depending on label directions. Aliquot in 0.5 ml amounts and store at or below -20°C. Each lot is supplied with a dilution titer. Since conditions and equipment differ from one laboratory to another, it is necessary to titer and test a new lot of conjugate with the fluorescent microscope assembly currently in use.[3,8,13]

1. Prepare serial dilutions in 2% Tween 80, including the titer specified on the vial.
2. Test each dilution per the Test Procedure with Reactive Control Serum (4+) and Nonspecific Staining Control.
3. Test a known lot of reagent using the Reactive Control Serum (4+), Minimally Reactive Control Serum (1+) and Nonspecific Staining Control as controls of the reagents and test conditions.
4. During further testing, use the dilution that produces 1 doubling dilution lower than the 4+ endpoint. The 4+ endpoint is the highest dilution of conjugate yielding 4+ fluorescence with the Reactive Control Serum (4+).

FA Buffer, Dried: Dissolve 10 grams in 1 liter of distilled or deionized water and rotate gently to completely dissolve the contents. Store at 2-8°C. Use the solution if it is free of mold growth and turbidity.

Tween® 80: Heat the bottle of Tween 80 and a flask containing 98 ml FA Buffer to 56°C in a water bath. Add 2 ml of Tween 80 to the buffer and rinse the pipette thoroughly in the buffer. Check the pH and adjust to pH 7.2 with 1N NaOH. Discard if a precipitate develops or the pH changes.

Specimen Collection and Preparation

Test serum: Collect patient (test) serum according to recommended procedures.[2,3,8,9,13] Store specimens at room temperature for up to 4 hours or at 2-8°C for up to 5 days; serum specimens may be frozen at or below -20°C.

Test and control sera: Equilibrate the sera to room temperature, then heat at 56°C for 30 minutes. Reheat previously heated sera for 10 minutes on the day of testing. Cool to room temperature before testing. Bacterial contamination or excessive hemolysis may render a specimen unsuitable for testing. Such specimens should not be tested.

Test Procedure

This procedure conforms with those published by the U. S. Department of Health, Education and Welfare[14] and with subsequent procedures published by the American Public Health Association.[9,13]

1. **FTA Antigen smears**: Obtain previously prepared smears, thaw and dry if appropriate, and identify the frosted end of the slides to correspond with each test and control serum to be tested.
2. Prepare the following test and control sera in appropriately identified tubes no more than 30 minutes before testing and mix thoroughly (at least 8 times):

   Test Serum (1:5): Dilute 0.05 ml (50 µl) of heated (or reheated) test serum in 0.2 ml (200 µl) FTA Sorbent.

   Reactive Control Serum (4+) - Unabsorbed: Dilute 0.05 ml (50 µl) FTA Serum Reactive in 0.2 ml (200 µl) FA Buffer (PBS).

   Reactive Control Serum (4+) - Absorbed: Dilute 0.05 ml (50 µl) FTA Serum Reactive in 0.2 ml (200 µl) FTA Sorbent.

   Minimally Reactive Control Serum (1+): Dilute FTA Serum Reactive, as indicated on the label, in FA Buffer (PBS) to yield a 1+ fluorescence. The minimal degree of fluorescence that can be reported as reactive is 1+ fluorescence.

   Nonreactive Control Serum (N) (1:40): Prepare a 1:40 dilution of FTA Serum Non-Reactive by adding 0.05 ml (50 µl) of serum to 1.95 ml FA Buffer (PBS).

   Nonspecific Serum Control - Unabsorbed (2+ nonspecif ic reactivity): Dilute 0.05 ml (50 µl) FTA Sorbent Control in 0.2 ml (200 µl) FA Buffer (PBS).

   Nonspecific Serum Control - Absorbed (nonreactive, - to ±): Dilute 0.05 ml (50 µl) FTA Sorbent Control in 0.2 ml (200 µl) FTA Sorbent.

   Nonspecific Staining Control - Unabsorbed: Use 0.03 µl (30 ml) FA Buffer (PBS) undiluted.

   Nonspecific Staining Control - Absorbed: Use 0.03 ml (30 µl) FTA Sorbent undiluted.

3. **FTA Antigen smears**: Cover the previously identified FTA Antigen smears with 0.03 ml (30 µl) of the corresponding test or control serum prepared above, making certain that the entire smear is covered.
4. Place the slides in a moist chamber to prevent evaporation and incubate at 35-37°C for 30 minutes.
5. Place the slides in a slide carrier and rinse as follows:
   - Rinse in running FA Buffer for 5 seconds.
   - Soak in FA Buffer for 5 minutes.
   - Agitate by dipping in and out of the buffer 30 times.
   - Repeat the soaking and agitation in fresh buffer.
   - Rinse in running distilled water for 5 seconds.
   - Gently blot dry with bibulous paper.
6. **FA Human Globulin Antiglobulin (Rabbit)**: Dilute the antiglobulin to its working titer (determined above) using 2% Tween 80 in FA Buffer.
7. **FTA Antigen smears**: Cover each test and control smear with approximately 0.03 ml (30 µl) of diluted FA Human Globulin Antiglobulin (Rabbit). Spread uniformly to cover the entire smear.
8. Repeat steps 4 and 5.
9. Mount the slides immediately using a small drop of FA Mounting Fluid pH 7.2 and apply a cover slip, being careful not to trap air bubbles in the mounting fluid.
10. Immediately examine the slides microscopically for intensity of fluorescence using the microscope assembly described above. If it is necessary to delay reading, store the slides in the dark and read within 4 hours. Results are valid only if the quality control pattern is satisfactory.
11. Verify the presence of treponemes on the nonreactive control slides by dark-field microscopy.

Results

Using the 1+ serum control as a reading standard, record the intensity of fluorescence of the treponemes and report as follows. Retest all specimens with an initial test fluorescence of 1+. When a specimen initially read as 1+ yields a retest reading of 1+ or greater, it is reported as reactive. All other results are reported as nonreactive. Retesting nonreactive specimens is not necessary.

Without historical or clinical evidence of treponemal infection, equivocal test results (see below) suggest the need for testing a second specimen obtained 1-2 weeks after the initial specimen.

INTENSITY OF FLUORESCENCE	INITIAL TEST RESULT	RETEST RESULT	REPORT
Moderate to strong	2+ to 4+	NA	Reactive
Equivalent to 1+ control	1+	>1+	Reactive
	1+	1+	Reactive minimal*
	1+	<1+	Nonreactive
Visible staining but <1+	± to <1+	NA	Nonreactive
None or vaguely visible, not distinct	–	NA	Nonreactive
"Moth eaten" or "beaded"			Atypical

*Equivocal result.

Limitations of the Procedure

1. When the treponemal test results and the clinical opinion disagree, repeat the treponemal test and obtain additional clinical and historical information. If the disagreement persists, send the specimen to a reference laboratory such as the local state health department for additional confirmatory tests. The final diagnosis depends on the clinical judgment of a specialist very experienced in sexually transmitted diseases.[2,3]
2. The test should not be used to follow the response to therapy nor can it be relied on to detect new, untreated cases in epidemiological investigations.
3. "Atypical" fluorescence and false-positive results have been associated with patients having active systemic, discoid and drug-induced varieties of lupus erythematosus[13-17] and other autoimmune diseases.
4. Elderly patients may exhibit unexplained FTA-ABS reactions.
5. At times, deciding whether a reading is weak or vaguely visible may be difficult. The ability to make this distinction is critical, since a nonreactive (vaguely visible to none) serum is not retested.

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Packaging

Product	Size	Cat. No.
FA Buffer, Dried	6 x 10 g	2314-33
	100 g	2314-15
FA Human Globulin Antiglobulin (Rabbit)	1 ml	2449-50
	5 ml	2449-56
FA Mounting Fluid pH 7.2	6 x 5 ml	2329-57
FTA Antigen	1 ml	2344-50
FTA Serum Non-Reactive	5 ml	2440-56
FTA Serum Reactive	5 ml	2439-56
FTA Sorbent	5 ml	3259-56
FTA Sorbent Control	6 x 0.5 ml	3266-49
Tween® 80	6 x 5 ml	3118-57

FTA-ABS Test Procedure

Abbreviated Schematic

STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
Prepare sera and reagents.	Dilute sera.	Add test and control sera to appropriate FTA Antigen smears.	Add conjugate to the FTA Antigen smears.	Record reactions of test and control sera. Verify that control sera provided the expected results.
FTA Antigen		**FTA Antigen Smear**		
Rehydrate with 1 ml distilled or deionized water. Prepare smears. Fix with acetone. Use as "FTA Antigen smear".		Thaw, dry and identify sufficient FTA Antigen smears to correspond with each of the test and control sera to be tested.		
Test (Patient) Serum				
Heat at 56°C for 30 minutes or reheat previously heated serum for 10 minutes.	Dilute 1:5 by adding 0.05 ml serum to 0.2 ml in FTA Sorbent.	Apply 0.03 ml test serum to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	Dependent on antibody status of test serum.
FTA Serum Reactive	**Reactive Control Serum (4+) – Unabsorbed**			
Rehydrate with 5 ml distilled or deionized water. Heat at 56°C for 30 minutes.	Dilute 1:5 by adding 0.05 ml FTA Serum Reactive to 0.2 ml FA Buffer.	Apply 0.03 ml Reactive Control Serum – Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	4+ Reactive
	Reactive Control Serum (4+) – Absorbed			
	Dilute 1:5 by adding 0.05 ml FTA Serum Reactive to 0.2 ml FTA Sorbent.	Apply 0.03 ml Reactive Control Serum – Absorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	3+ to 4+ Reactive
	Minimally Reactive Control Serum (1+)			
	Dilute FTA Serum Reactive to 1+ fluorescence (labeled titer) in FA Buffer.	Apply 0.03 ml Minimally Reactive Control Serum to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	1+ Reactive

FTA Serum Nonreactive	Nonreactive Control Serum			
Rehydrate with 5 ml distilled or deionized water. Heat at 56°C for 30 minutes.	Dilute 1:40 by adding 0.05 ml FTA Serum Nonreactive to 1.95 ml FA Buffer.	Apply 0.03 ml Nonreactive Control Serum to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N Nonreactive

FTA Sorbent Control	Nonspecific Serum Control – Unabsorbed			
Rehydrate with 0.5 ml distilled or deionized water. Heat at 56°C for 30 minutes.	Dilute 1:5 by adding 0.05 ml FTA Sorbent Control to 0.2 ml FA Buffer.	Apply 0.03 ml Nonspecific Serum Control - Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	2+ to 4+ Reactive
	Nonspecific Serum Control – Absorbed			
	Dilute 1:5 by adding 0.05 ml FTA Sorbent Control to 0.2 ml FA Buffer.	Apply 0.03 ml Nonspecific Serum Control - Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N to ± Nonreactive

FA Buffer, Dried	Nonspecific Staining Control – Unabsorbed			
Dissolve 10 grams in 1 liter distilled or deionized water.	Use 0.03 ml FA Buffer 0.05 ml FTA Serum Nonreactive to 1.95 ml FA Buffer.	Apply 0.03 ml Nonspecific Staining Control - Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N Nonreactive

FTA Sorbent	Nonspecific Staining Control – Absorbed			
Rehydrate with 5 ml distilled or deionized water.	Use 0.03 ml FTA Sorbent undiluted as the diluent (above) and as the Nonspecific Staining Control - Absorbed	Apply 0.03 ml Nonspecific Staining Control -Absorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N Nonreactive

FA Human Globulin Antiglobulin (Rabbit)	
Rehydrate with 1 ml or 5 ml distilled or deionized water. Determine titer if a new lot.	Dilute to labeled titer with 2% Tween. Use as "Conjugate".

Tween® 80
Heat Tween 80 and FA Buffer to 56°C. Add 2 ml Tween 80 to 98 ml FA Buffer. Adjust to pH 7.2.

Bacto® Febrile Antigen Set

Contains: Brucella Abortus Antigen (Slide) · Proteus OX19 Antigen (Slide) · Salmonella O Antigen Group D · Salmonella H Antigen a Salmonella H Antigen b · Salmonella H Antigen d · Febrile Positive Control Polyvalent · Febrile Negative Control

Intended Use

Bacto Febrile Antigen Set is used in the detection of febrile antibodies by the slide and tube agglutination tests.

Summary and Explanation

Agglutination tests have been used in diagnosing certain febrile illnesses since the early 1900's. Patients experiencing "febrile" symptoms, including fever, chills, malaise and fatigue, were considered likely to have typhoid fever, brucellosis, rickettsial infection (either typhus or spotted fever) or tularemia. The agents of these infections are difficult or unlikely to be isolated by routine laboratory methods but do cause detectable increases in antibody levels in the patient's serum.

User Quality Control

Identity Specifications

Brucella Abortus Antigen (Slide), Proteus OX19 Antigen (Slide), Salmonella O Antigen Group D, Salmonella H Antigen a, Salmonella H Antigen b, Salmonella H Antigen d

Appearance: Turquoise-blue-violet suspension.

Febrile Positive Control Polyvalent

Lyophilized appearance: Light gold to amber, button to powdered cake.

Rehydrated appearance: Light gold to amber, clear liquid.

Febrile Negative Control

Lyophilized appearance: Colorless to light gold, button to powdered cake.

Rehydrated appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Febrile Positive Control Polyvalent and Febrile Negative Control per label directions. Perform the slide or tube agglutination test using an appropriate Febrile Antigen and positive and negative controls diluted in the same proportion as a patient serum.

A Febrile Antigen is considered satisfactory if it does not agglutinate with the negative control and shows 2+ or greater agglutination with the positive control at the following dilution:

Brucella Abortus Antigen	1:80
Proteus OX19 Antigen	1:160
Salmonella O Antigen Group D	1:80
Salmonella H Antigen a	1:80
Salmonella H Antigen b	1:80
Salmonella H Antigen d	1:80

"Febrile Antigen" is a term generally referring to bacterial suspensions representative of many species of microorganisms pathogenic to man which are accompanied by a fever in the host. A battery of febrile antigens evolved as the "febrile antigen" or "febrile agglutinin" test. The febrile antigen test is based on the Widal test (*Salmonella* somatic O and flagellar H antigens), the Weil-Felix test (antigens of selected *Proteus* strains), and the *Brucella abortus* antigen test.[1,2,3] In some situations, the *Francisella tularensis* antigen test is included in the battery.

DISEASE	ASSOCIATED FEBRILE ANTIGEN
Brucellosis	Brucella abortus
Rocky Mountain spotted fever	Proteus OX19
Typhus	Proteus OX19
Typhoid fever	Salmonella O Antigen Group D
Typhoid fever	Salmonella H Antigen d
Paratyphoid fever	Salmonella H Antigen a
Paratyphoid fever	Salmonella H Antigen b

In 1896, Widal introduced techniques for testing patients' serum for antibodies in cases of typhoid fever.[1] The Widal test was used diagnostically in two ways. First, it was considered diagnostic when a single high titer of antibodies occurred during the first week of illness. In addition, it was diagnostic if a greater than fourfold titer rise existed in serum samples taken 1 to 2 weeks apart.[2,4,5,6] The Widal test was developed to include *Salmonella typhi* and other species of *Salmonella* detected by a variety of O and H antigens. *S. typhi* and *S. paratyphi* A and B are the major pathogens in this group that can produce clinically distinct systemic illness. The Widal test for antibodies to the O antigens of *Salmonella* serotypes most likely to cause typhoid fever (usually *S. typhi* and *S. paratyphi* A and B) can be useful in diagnosing typhoid fever when other methods have failed.[7]

The Weil-Felix test became popular in the 1920's after it was observed that certain strains of *Proteus* would agglutinate early convalescent-phase sera from patients with suspected rickettsial disease.[3] *Proteus* antigens (OX2, OX19 and OXK) will cross-react in predictable patterns, although the reactions are not highly sensitive or specific.

Diagnosis of the cause of febrile disease cannot be based solely on the analysis of serum samples for antibody response. Many factors may affect measurable antibody levels. For example, the patient's immune response can be affected by age, immune status, general state of health and previous immunizations.

Certain organisms may share cross-reacting antigens leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in an antibody test procedure resulting in low-level antibody titers that may not, when used alone, suggest disease. Cross reactions can occur among species of *Francisella* and *Brucella*, among various species of *Salmonella*, and

between *Brucella* species and *Yersinia enterocolitica* or *Vibrio cholerae*. Antibodies produced in response to a *Proteus* infection can react with *Proteus* OX19 and be misinterpreted as rickettsial antibodies.

The rapid slide test is the most widely used procedure employing febrile antigens because of the simplicity with which the results may be reported. Negative slide test reactions can usually be reported as such if all five serum dilutions have been used. Although the slide test is not quantitative, running the series of dilutions is necessary to detect agglutinin content of a serum that might be overlooked for a "prozone phenomenon" where higher concentrations of the serum may yield negative results, but a dilution of the serum is positive. This often occurs in sera containing *Brucella* agglutinins and, to a lesser extent, typhoid agglutinins.

The macroscopic tube test[2] should be used to confirm the presence of antibodies demonstrated by the slide technique and to quantitate their titer in suspect sera. When quantitative determinations of *Rickettsia* or *Brucella* agglutinins are necessary, tube antigens are used.

Principles of The Procedure

Agglutination tests involving the use of febrile antigens determine the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum, then adding a standard volume of antigen. The end point of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point converted to a dilution of the serum is called the patient's antibody "titer."

Reagents

Antigens

1. Febrile Antigens are ready-to-use, whole cell suspensions of the organisms listed below. Proteus OX19 Antigen (Slide) contains 20% glycerin.
   Brucella Abortus Antigen (Slide) - *Brucella abortus*
   Proteus OX19 Antigen (Slide) - *Proteus vulgaris* OX19
   Salmonella O Antigen Group D - *Salmonella typhi* O901
   Salmonella H Antigen a - *Salmonella paratyphi* A
   Salmonella H Antigen b - *Salmonella paratyphi* B
   Salmonella H Antigen d - *Salmonella typhi* H901
2. **Slide test**: The Febrile Antigens (Brucella Abortus Antigen (Slide), Proteus OX19 Antigen (Slide), Salmonella O Antigen Group D, Salmonella H Antigen a, Salmonella H Antigen b and *Salmonella typhi* H901) are used in the slide test and contain sufficient reagent for 20 slide tests.

   Tube test: Salmonella O and H Antigens may also be used in the tube test and contain sufficient reagent for 25 tube tests.

   Brucella Abortus Antigen (Slide) and Proteus OX19 Antigen (Slide) are used *only* in the slide test. When confirmation of the slide test and quantitation are required, Brucella Abortus Antigen (Tube) and Proteus OX19 (Tube) may be purchased as separate products.
3. **Antigen Density**: Salmonella O and H Antigens are adjusted to a density approximating 20 times a McFarland Barium Sulfate Standard No. 3 (1.8 x 10^{10} organisms per ml). These antigens are used undiluted for the slide test and diluted 1:20 for the tube test.

   Because antigen density may vary, it is adjusted for optimum performance when standardized with hyperimmune sera obtained from laboratory animals.

   Variation in antigen color intensity is normal and will not affect test performance.
4. Febrile Antigens contain the following preservatives:

   Brucella Abortus Antigen (Slide): 0.5% phenol, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Proteus OX19 Antigen (Slide): 0.25% formaldehyde, and approximately 0.002% crystal and 0.005% brilliant green.

   Salmonella O Antigen Group D: 0.5% phenol, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella H Antigens a: 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella H Antigens b: 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella H Antigens d: 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

Antisera

1. **Febrile Positive Control Polyvalent** is lyophilized, polyclonal, polyvalent goat antisera containing approximately 0.04% Thimerosal as a preservative. It contains antibodies for all of the components of the Febrile Antigen Set. Each vial contains sufficient reagent for 32 slide tests or 50 tube tests using single antigens or for approximately 5 slide tests when using all of the antigens in the set.
2. **Febrile Negative Control** is a lyophilized, standard protein solution containing approximately 0.02% Thimerosal as a preservative. Each vial contains sufficient reagent for 32 slide tests using single antigens or for approximately 5 slide tests using all of the antigens in the set.

Precautions

1. For In Vitro Diagnostic Use.
2. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[8,9]
3. **Proteus OX19 Antigen (Slide)**
   Salmonella H Antigen a
   Salmonella H Antigen b
   Salmonella H Antigen d
   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Kidneys, Lungs, Skin.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.
5. Febrile Antigens are not intended for use in the immunization of humans or animals.

Storage

Store Febrile Antigens at 2-8°C.

Store lyophilized and rehydrated Febrile Positive Control Polyvalent at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to a product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Febrile Antigen Set:

Brucella Abortus Antigen (Slide)
Proteus OX19 Antigen (Slide)
Salmonella O Antigen Group D
Salmonella H Antigen a
Salmonella H Antigen b
Salmonella H Antigen d
Febrile Positive Control Polyvalent
Febrile Negative Control

Materials Required But Not Provided

Slide Test

Agglutination slides, 5 squares, 1" each
Applicator sticks
Sterile deionized water or equivalent
Serological pipettes, 0.2 ml

Tube Test

Culture tubes 12 x 75 mm and rack
Waterbath, 35-37°C and 50 ± 2°C
Refrigerator, 2-8°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution

Reagent Preparation

Febrile Antigens are ready to use.

Febrile Positive Control Polyvalent: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Febrile Negative Control: To rehydrate, add 5 ml sterile deionized water, or equivalent, and rotate gently to completely dissolve the contents.

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergent.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. Serum is required for the test. Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain at or below -20°C. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Refer to appropriate references for more information on collection of specimens.[10,11] Serum specimens must not be heated. Heat may inactivate or destroy certain antibodies.

An increase in titer over a period of time is the best indicator of active infection. The accuracy and precision of the tests can be affected not only by test conditions, but also by the subjectivity of the person reading the endpoint.

A preliminary test using either the rapid slide test and/or the macroscopic tube test may be performed on the initial serum specimen and reported to the physician at that time. An aliquot of the serum should be transferred to a sterile test tube, sealed tightly, and kept in the freezer. When the second serum is obtained, it should be run in parallel with the original specimen. In this manner, the original serum will serve as a control and any difference in titer will be more credible, since the bias associated with the performance of the test and determining the endpoint will be reduced.

Test Procedure

Slide Test

Use the slide test only as a screening test; confirm positive results with the tube test. Test each Febrile Antigen separately, repeating steps 1-6 for each Antigen.

1. **Test Serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of each test serum into a row of squares on the agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Positive Control Polyvalent into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Febrile Antigen**: Gently shake the vial of antigen to ensure a smooth, uniform suspension. Place one drop (35 µl) of antigen suspension in each drop of test serum, positive control and negative control.
5. Mix each row of test and control serum, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond with tube dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, respectively.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 2+ or greater agglutination at the following dilutions:

Brucella Abortus Antigen	1:80
Proteus OX19 Antigen	1:160
Salmonella O Antigen Group D	1:80
Salmonella H Antigen a	1:80
Salmonella H Antigen b	1:80
Salmonella H Antigen d	1:80

3. **Negative control**: Should show no agglutination.
4. **Test specimens**: The serum titer is that dilution which shows 2+ or greater agglutination. See Table 1.

Table 1. Sample Rapid Slide Test reactions.

SERUM (ml)	CORRELATED TUBE DILUTION	REACTIONS		
		SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
0.08	1:20	3+	4+	4+
0.04	1:40	2+	4+	3+
0.02	1:80	1+	3+	2+
0.01	1:160	–	3+	+
0.005	1:320	–	1+	–
Serum titer		**1:40**	**1:160**	**1:80**

Tube Test

Salmonella O Antigen Group D and Salmonella H Antigens a, b and d in the Febrile Antigen Set are used for both slide and tube agglutination tests. Brucella Abortus Antigen (Slide) and Proteus OX19 Antigen (Slide) are intended only for slide tests. When confirmation of the slide test and quantitation is required, separate tube test antigens, Brucella Abortus Antigen (Tube) and Proteus OX19 (Tube), may be purchased separately.

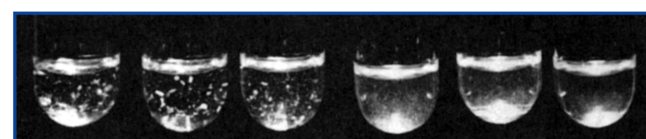
Each Febrile Antigen must be tested separately. Repeat steps 1-10 for each antigen.

Prepare a 1:20 dilution of each antigen to be tested by adding 1 part of antigen to 19 parts of sterile NaCl solution.

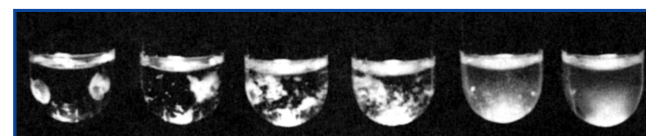
1. Prepare a row of 8 culture tubes (12 x 75 ml) for each test serum, including a row for the Febrile Positive Control Polyvalent.
2. **0.85% NaCl solution**: Dispense 0.9 ml in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, dispense 0.1 ml of test serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
4. **Positive control**: Using a 1 ml serological pipette, dispense 0.1 ml of Febrile Positive Control Polyvalent in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
5. **Febrile Antigen**: Add 0.5 ml of the diluted antigen suspension to all 8 tubes in each row and shake the rack to mix.
6. The final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
7. Incubate as specified (eg., in a waterbath or refrigerator):
   Brucella Abortus Antigen: 35-37°C for 48 ± 3 hours.
   Proteus OX19 Antigen: 35-37°C for 2 hours, then at 2-8°C for 22 ± 2 hours.
   Salmonella O Antigen Group D: 50 ± 2°C for 17 ± 1 hours.
   Salmonella H Antigens a: 50 ± 2°C for 1 hour.
   Salmonella H Antigens b: 50 ± 2°C for 1 hour.
   Salmonella H Antigens d: 50 ± 2°C for 1 hour.
8. Remove from incubation. Avoid excessive shaking before reading the reactions, either when the tubes are incubating or when removing them from incubation.
9. Read and record results.

Results

1. Read and record results as follows.
   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.
2. Salmonella Antigens used in tube agglutination procedures detect antibodies to either O (somatic) antigens or H (flagellar) antigens and these antibodies give different reactions. An O antigen and the corresponding antibody give a coarse, compact agglutination that may be difficult to disperse. An H antigen and its corresponding antibody give a loose flocculent agglutination. Do not vigorously shake tubes containing H antigens. Characteristic O and H agglutination is illustrated below.



Somatic "O" Agglutination



Flagellar "H" Agglutination

3. **Positive control**: Should show a 2+ or greater agglutination at the following dilutions:
   Brucella Abortus Antigen 1:80
   Proteus OX19 Antigen 1:160
   Salmonella O Antigen Group D 1:80
   Salmonella H Antigens a, b and d 1:80
4. **Antigen control**: Tube 8 of each row should show no agglutination.
5. **Test serum**: The serum titer is that dilution which shows a 2+ or greater agglutination. See Table 2.

Table 2. Sample Macroscopic Tube Test reactions.

SERUM DILUTION	REACTIONS		
	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
1:20	4+	3+	4+
1:40	4+	2+	4+
1:80	3+	1+	4+
1:160	2+	–	4+
1:320	1+	–	3+
1:640	–	–	2+
1:1280	–	–	1+
Serum titer	**1:160**	**1:40**	**1:640**

Interpretation

1. Compare results:

DISEASE	ASSOCIATED FEBRILE ANTIGEN	SIGNIFICANT TITER
Brucellosis	Brucella Abortus	1:160
Rocky Mountain spotted fever*	Proteus OX19	1:160
Typhus*	Proteus OX19	1:160
Typhoid fever	Salmonella O Antigen Group D**	1:80
Typhoid fever	Salmonella H Antigen d**	1:80
Paratyphoid fever	Salmonella H Antigen a**	1:80
Paratyphoid fever	Salmonella H Antigen b**	1:80

* Rocky Mountain spotted fever cannot be differentiated from typhus by this test.

** Antibodies produced in response to other *Salmonella* species can cross-react.

2. **Single serum specimen**: A significant titer suggests infection.
3. **Pair of serum specimens (acute and convalescent)**: A two-dilution increase in titer is significant and suggests infection. A one-dilution difference is within the limits of laboratory error.
4. **Positive control and antigen control**: If results are not as described, the test is invalid and results cannot be reported.

Limitations of the Procedure

1. The slide test is intended for screening only and should be confirmed by the tube test. Slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all 5 serum dilutions in the slide test should be run.
2. Detection of antibodies in serum specimens may complete the clinical picture of a patient having an infection. However, isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician based on patient history, physical examination and data from all laboratory tests.
3. Cross-reacting heterologous antibodies are responsible for many low-titer reactions. Infections with other organisms, vaccinations and history of disease may result in a low level of antibody titer. Antimicrobial therapy may suppress antibody production.

   Cross reactions between antigens and antibodies of *B. abortus* and *F. tularensis*, *Y. enterocolitica* or *V. cholerae* can occur.

   Rocky Mountain spotted fever and typhus cannot be differentiated by this test because species of *Rickettsia* cause cross-reacting antibodies.

   Infections with *Proteus* species can cause cross-reacting antibodies.

   Cross-reactions between antigens and antibodies of various *Salmonella* species can occur.

   Previous immunizations with typhoid vaccine or previous infection with *Salmonella* species sharing common antigens with *S. typhi* can cause elevated antibody titers for prolonged periods. Other non-typhoid febrile illnesses may cause elevation of cross-reacting antibodies.
4. While a single serum specimen showing a significant titer suggests infection, it is not diagnostic.
5. To test for a significant rise in antibody titer, at least two specimens are necessary: an acute specimen, obtained at the time of initial symptoms, and a convalescent specimen, obtained 7 to 14 days later. A two-dilution difference in the titers is a significant increase in antibody level and suggests infection.
6. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
7. Exposure to temperatures below 2°C can cause autoagglutination. Antigens must be smooth, uniform suspensions; before use, examine antigen vials for agglutination. Suspensions with agglutination are not usable and should be discarded.
8. Discard rehydrated Febrile Positive Control Polyvalent or Febrile Negative Control that is cloudy or has a precipitate anytime during its period of use.

References

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11. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection, transport and storage. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Febrile Antigen Set	8 x 5 ml	2407-32
Contains:		
Brucella Abortus Antigen (Slide)		
Proteus OX19 Antigen (Slide)		
Salmonella O Antigen Group D		
Salmonella H Antigen a		
Salmonella H Antigen b		
Salmonella H Antigen d		
Febrile Positive Control Polyvalent		
Febrile Negative Control		

Available separately:

Brucella Abortus Antigen (Slide)	5 ml	2909-56
Brucella Abortus Antigen (Tube)	25 ml	2466-65
Proteus OX19 Antigen (Slide)	5 ml	2234-56
Proteus OX19 Antigen (Tube)	25 ml	2247-65
Salmonella O Antigen Group D	5 ml	2842-56
Salmonella H Antigen a	5 ml	2844-56
Salmonella H Antigen b	5 ml	2845-56
Salmonella H Antigen d	5 ml	2847-56
Febrile Positive Control Polyvalent	5 ml	3238-56
Febrile Negative Control	5 ml	3239-56

Bacto® Francisella Tularensis Antigens and Antisera

Francisella Tularensis Antigen (Slide) · Francisella Tularensis Antigen (Tube) · Francisella Tularensis Antiserum Febrile Negative Control

Intended Use

Bacto Francisella Tularensis Antigens (Slide) and (Tube) are used in the detection of antibodies by the slide and tube agglutination tests.[1,2]

Bacto Francisella Tularensis Antiserum is used to demonstrate a positive quality control test reaction in the slide and tube agglutination tests.

Bacto Febrile Negative Control is used to demonstrate a negative quality control test reaction in the slide agglutination test.

Summary and Explanation

Two species of the genus *Francisella* exist, *Francisella tularensis* and *Francisella novicidia*.[3] The latter species occurs rarely and is not known to infect humans.

F. tularensis is the causative agent of tularemia in humans. The disease was first described in humans in 1907.[1] It is a zoonotic disease transmitted to humans by direct contact with wild animals or bites of insect vectors such as ticks and biting flies. Wild animals such as rabbits, beavers, muskrats, domestic mammals and birds are involved in disease transmission.

The organism directly invades the skin, conjunctiva or mucosa of the oropharynx from blood or tissue of the infected animal. Indirect transmission includes bites of insect vectors, inhalation of contaminated feces or soil, or ingestion of contaminated water or poorly cooked meat.

Patients experience a rapid onset of "febrile" symptoms including malaise, chills, fever and fatigue. Several forms of the infection occur, each with additional characteristic symptoms. *F. tularensis* is a pathogenic microorganism that, upon invasion, produces a fever in its host. Consequently, it is often called a "Febrile Antigen."

For growth on culture media, *F. tularensis* requires both blood and cystine or cysteine. Gram stains of cultural isolates aid in the identification of the organism. The organisms are gram negative, stain faintly, and have extremely small coccoid cells that are often hard to visualize even at 1,000X magnification.[2]

The human immune response to a particular microorganism results in measurable antibody production that can sometimes help in completing the patient's clinical diagnosis. In blood samples, the antibody titer during the initial (acute) phase of the infection is compared to the antibody titer 7-14 days later (convalescent). Antibody titers that are high initially in the acute phase (1:160) or an acute or con valescent pair of samples that shows an increase in antibody titer are helpful in the diagnosis of tularemia.[4,5,6]

Diagnosis of the cause of febrile disease cannot be based solely on the analysis of serum samples for antibody response. Many factors may affect measurable antibody levels. For example, the patient's immune response can be affected by age, immune status, general state of health and previous immunizations.

Certain organisms may share cross-reacting antigens, leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in a febrile antibody test procedure, producing low-level antibody titers. A titer of less than 1:20 is not considered diagnostic because nonspecific cross-reactions are common at this level.[1] Cross-reactions between *Francisella* and *Brucella* can occur.

Principles of the Procedure

Agglutination tests involving the use of *Francisella* antigens detect the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum and then adding a standard volume of antigen. The endpoint of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point, reported as a dilution of the serum, is called the patient's antibody "titer."

Reagents

Francisella Tularensis Antigen (Slide) is a ready-to-use suspension of *Francisella tularensis* containing 20% glycerin, as well as 0.5% phenol, approximately 0.2% crystal violet and approximately 0.5% brilliant green as preservatives. When used as described, each 5 ml vial contains sufficient reagent for 20 slide tests.

Francisella Tularensis Antigen (Tube) is a ready-to-use suspension of *Francisella tularensis* adjusted to a density approximating a McFarland Barium Sulfate Standard No. 3 (9×10^8 organisms per ml). Francisella Tularensis Antigen (Tube) contains 0.5% formalin but does not contain dye. When used as described, each 25 ml vial contains sufficient reagent for 6 tests.

Because antigen density may vary, density is adjusted to ensure optimum performance when the antigen is standardized with hyperimmune sera obtained from laboratory animals. Variation in antigen color intensity is normal and will not affect the outcome of the test.

Francisella Tularensis Antiserum is a lyophilized, polyclonal rabbit antiserum containing approximately 0.04% Thimerosal as a preservative. When rehydrated and used as described, each 3 ml vial contains sufficient reagent for 19 slide tests or 30 tube tests.

Febrile Negative Control is a standard protein solution containing approximately 0.02% Thimerosal as a preservative. When used as described, each 3 ml vial contains sufficient reagent for 32 slide tests.

User Quality Control

Identity Specifications

Francisella Tularensis Antigen (Slide)
Appearance: Blue-violet suspension.

Francisella Tularensis Antigen (Tube)
Appearance: Light gray to white suspension.

Francisella Tularensis Antiserum
Lyophilized Appearance: Light gold to amber, button to powdered cake.
Rehydrated Appearance: Light gold to amber, clear liquid.

Febrile Negative Control
Lyophilized Appearance: Colorless to light gold, button to powdered cake.
Rehydrated Appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Francisella Tularensis Antiserum and Febrile Negative Control per label directions. Perform the Rapid Slide Test using Francisella Tularensis Antigen (Slide) or the Macroscopic Tube Test using Francisella Tularensis Antigen (Tube). Dilute both positive and negative controls in the same proportion as a patient serum and process in the same manner, following appropriate procedure.

An antigen is considered satisfactory if it fails to agglutinate with the negative control and reacts to a titer of 1:160 or more with the positive control.

Precautions

1. For In Vitro Diagnostic Use.
2. **Francisella Tularensis Antigen (Tube)**
   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Eyes, Kidneys, Lungs, Skin.
   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[7,8]
4. Biosafety level 2 precautions are recommended when handling specimens suspected of containing *F. tularensis*.[9]
5. Francisella Tularensis Antigens are not intended for use in the immunization of humans or animals.
6. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Francisella Tularensis Antigens (Slide) and (Tube) at 2-8°C.

Store lyophilized and rehydrated Francisella Tularensis Antiserum at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Francisella Tularensis Antigen (Slide)
Francisella Tularensis Antigen (Tube)
Francisella Tularensis Antiserum
Febrile Negative Control

Materials Required But Not Provided

Slide Test

Agglutination slides with five 1-inch squares
Applicator sticks
Sterile 0.85% NaCl solution
Serological pipettes, 0.2 ml
Distilled or deionized water

Tube Test

Culture tubes, 12 x 75 mm, and rack
Waterbath, 35-37°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution
Distilled or deionized water

Reagent Preparation

Francisella Tularensis Antigen (Slide) and Francisella Tularensis Antigen (Tube) are ready to use.

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

Francisella Tularensis Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to dissolve the contents completely. The rehydrated antiserum is considered a 1:2 working dilution.

Febrile Negative Control: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to dissolve the contents completely.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. After the specimen has clotted, centrifuge to obtain the serum required for the test. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Consult appropriate references for more information on collection of specimens.[2,10]

Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain below -20°C. Serum specimens must not be heated; heat may inactivate or destroy certain antibodies.

Slide Test

Use the slide test only as a screening test. Confirm positive results with the tube test.

1. **Test serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of each test serum into a row of squares on an agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Francisella Tularensis Antiserum into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Antigen**: Shake the vial of Francisella Tularensis Antigen (Slide) thoroughly to ensure a smooth, uniform suspension. Dispense 1 drop (35 μl) of antigen in each drop of test serum, positive control and negative control.
5. Mix each row of test serum and control serum, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond approximately to tube dilutions of 1:20, 1:40, 1:80, 1:160 and 1:320, respectively.

Tube Test

1. In a rack, prepare a row of 8 culture tubes (12 x 75 mm) for each test serum and a positive control row for the Francisella Tularensis Antiserum.
2. Dispense 0.9 ml of sterile 0.85% NaCl solution in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, dispense 0.1 ml of serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Proceed in like manner for each serum to be tested.
4. **Positive control**: Using a 1 ml serological pipette, dispense 0.1 ml of Francisella Tularensis Antiserum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing.
5. **Antigen control**: Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
6. **Antigen**: Shake the vial of Francisella Tularensis Antigen (Tube) to ensure a smooth, uniform suspension. Add 0.5 ml of antigen to all 8 tubes in each row and shake the rack to mix the suspensions.
7. Final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
8. Incubate in a waterbath at 35-37°C for 22 ± 2 hours.
9. Remove from the waterbath. Avoid excessive shaking before reading the reactions, when the tubes are in the waterbath, or when removing them from the waterbath.

Results

1. Read and record results as follows.

   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.

2. **Positive control**: Should produce 2+ or greater agglutination at a 1:160 dilution.

   Negative control - Rapid Slide Test, only: Should produce no agglutination.

   Antigen control - Macroscopic Tube Test, only: Should produce no agglutination in tube #8 of each row.

   If results for either the positive or negative control are not as specified, the test is invalid and results cannot be reported.

   Test serum: The titer is the highest dilution that shows 2+ agglutination.

   Refer to Table 1 and Table 2[1] for examples of test reactions.

3. The Rapid Slide Test is a screening test, only; results must be confirmed using the Macroscopic Tube Test.

Table 1. Sample Rapid Slide Test reactions.

SERUM (ml)	CORRELATED TUBE DILUTION	REACTIONS		
		SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
0.08	1:20	3+	4+	4+
0.04	1:40	2+	4+	3+
0.02	1:80	1+	3+	2+
0.01	1:160	–	3+	+
0.005	1:320	–	1+	–
Serum titer		**1:40**	**1:160**	**1:80**

Table 2. Sample Macroscopic Tube Test reactions.

SERUM DILUTION	REACTIONS		
	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
1:20	4+	3+	4+
1:40	4+	2+	4+
1:80	3+	1+	4+
1:160	2+	–	4+
1:320	1+	–	3+
1:640	–	–	2+
1:1280	–	–	1+
Serum titer	**1:160**	**1:40**	**1:640**

Interpretation

For a single serum specimen, a titer of 1:160 at 2+ or greater suggests infection.[1]

A 2-dilution increase in the titer of paired serum specimens (from the acute to the convalescent serum) is significant and suggests infection. A 1-dilution difference is within the limits of laboratory error.

Limitations of the Procedure

1. The slide test is intended for screening only and results should be confirmed by the tube test. Slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all five serum dilutions (slide test) should be run.
2. The detection of antibodies in serum specimens may complete the clinical picture of tularemia. However, isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician based on patient history, physical examination and data from all laboratory tests.
3. Cross-reacting heterologous antibodies are responsible for many low titer reactions. Cross-reactions between antigens and antibodies of *Brucella* species and *Francisella tularensis* can occur. Infections with other organisms, vaccinations and a history of disease may cause low antibody titers. Antimicrobial therapy may suppress antibody production.
4. While a single serum specimen showing a titer of 1:160 suggests infection, it is not diagnostic.
5. To test for a significant rise in antibody titer, at least two specimens are necessary, an acute specimen obtained at the time of initial symptoms and a convalescent specimen obtained 7 to 14 days later. A two-dilution increase in titer is significant and suggests infection.
6. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
7. Exposure to temperatures below 2°C can cause antigen autoagglutination. Antigens must be smooth, uniform suspensions. Examine antigen vials for agglutination before use. Agglutinated suspensions are not usable and should be discarded.
8. Adhering to the recommended time and temperature of incubation is important when performing this test. For best results, locate the waterbath in an area free of mechanical vibration.

References

1. **Stewart, S. J.** 1995. *Francisella*, p. 545-548. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
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Packaging

Francisella Tularensis Antigen (Slide)	5 ml	2240-56
Francisella Tularensis Antigen (Tube)	5 ml	2251-56
	25 ml	2251-65
Francisella Tularensis Antiserum	3 ml	2241-47
Febrile Negative Control	3 ml	3239-56

Bacto® Haemophilus Influenzae Antisera

Haemophilus Influenzae Antiserum Poly · Haemophilus Influenzae Antiserum Type a · Haemophilus Influenzae Antiserum Type b · Haemophilus Influenzae Antiserum Type c Haemophilus Influenzae Antiserum Type d · Haemophilus Influenzae Antiserum Type e · Haemophilus Influenzae Antiserum Type f

Intended Use

Bacto Haemophilus Influenzae Antisera are used in slide agglutination tests for serotyping *Haemophilus influenzae*.

Summary and Explanation

H. influenzae was first described by Pfeiffer[1] in 1892 from patients during an influenza pandemic. Pittman[2] described the six capsular serotypes of *H. influenzae* in 1931. He recognized that members of serotype b were most likely to cause invasive infections.

H. influenzae is part of the normal respiratory flora of humans and many animal species. Often, the organism becomes an opportunistic secondary invader, usually following viral influenza. This organism can cause a variety of diseases from chronic respiratory infections to meningitis. Most of the *H. influenzae* isolates associated with meningitis possess the serotype b capsule.[3] Serotype b is believed to cause more than 90% of all *Haemophilus* infections in children less than six years of age. Although the incidence of *H. influenzae* type b infections has been drastically reduced by the introduction of effective vaccines, *Haemophilus* species remain important causes of a wide range of human infections.

User Quality Control

Identity Specifications

Haemophilus Influenzae Antiserum Poly
Haemophilus Influenzae Antiserum Type a
Haemophilus Influenzae Antiserum Type b
Haemophilus Influenzae Antiserum Type c
Haemophilus Influenzae Antiserum Type d
Haemophilus Influenzae Antiserum Type e
Haemophilus Influenzae Antiserum Type f

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber liquid.

Performance Response

Rehydrate Haemophilus Influenzae Antisera per label directions. Test as described (see Test Procedure). Known positive and negative control cultures must give appropriate reactions.

H. influenzae is a nonmotile, facultative anaerobe requiring both factor X (hemin) and factor V (NAD) for in vitro growth. In microscopic morphology, the organism is a pleomorphic gram-negative coccobacillus and sometimes forms threads or filaments.

The presence of a polysaccharide capsule is a major virulence factor for strains of *H. influenzae* that cause systemic infection. *H. influenzae* is divided into serological groups a, b, c, d, e and f based on capsular polysaccharides. Most encapsulated strains that cause infection belong to serotype b.[1] The encapsulated strains are referred to as typeable strains. Nonencapsulated or non-typeable strains may also cause infection. Infections caused by nonencapsulated strains are usually related to the upper respiratory tract.

Antigenic similarities exist between *H. influenzae* and many unrelated bacteria. *H. influenzae* serotype b shares cross-reacting antigens with *Streptococcus pneumoniae* serotypes 6, 15a, 29 and 35a, *Escherichia coli*, and several species of *Staphylococcus*, *Streptococcus and Bacillus.*

The Quellung (swelling) reaction has also been used for recognition of encapsulated (typeable) strains of *H. influenzae*.[1,4] The principle of this antigen-antibody reaction is not agglutination as in the slide technique, but an apparent increase in capsular size due to deposition of antibody on the cell surface. If the Quellung reaction is performed, one must be aware that these organisms are often found in the nonencapsulated state, which are untypable. In addition, capsulated strains of type "e" generally possess small capsules. Such strains should be defined serologically employing the slide agglutination test, only. Consult an appropriate reference for details of the Quellung reaction.[4]

Principles of the Procedure

Identification of *H. influenzae* includes isolation of the microorganism, biochemical identification and serological confirmation.

Serological confirmation involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. This *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity), and binds (high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to other species, heterologous reactions are possible. These are weak in strength or slow in formation. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous

agglutination reaction should support the morphological and biochemical identification of the microorganism.

Homologous reactions are rapid and strong. Heterologous reactions are slow and weak.

Reagents

Haemophilus Influenzae Antisera are lyophilized, polyclonal rabbit antisera containing approximately 0.02% Thimerosal as a preservative.

When rehydrated and used as described, each 1 ml vial of Haemophilus Influenzae Antiserum contains sufficient reagent for 20 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Haemophilus Influenzae Antisera at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Haemophilus Influenzae Antiserum Poly
Haemophilus Influenzae Antiserum Type a
Haemophilus Influenzae Antiserum Type b
Haemophilus Influenzae Antiserum Type c
Haemophilus Influenzae Antiserum Type d
Haemophilus Influenzae Antiserum Type e
Haemophilus Influenzae Antiserum Type f

Materials Required but not Provided

Agglutination slides
Applicator sticks
Sterile distilled or deionized water
Sterile 0.85% NaCl solution

Reagent Preparation

Haemophilus Influenzae Antiserum: To rehydrate, add 1 ml sterile distilled or deionized water and rotate to completely dissolve the contents.

Equilibrate all materials to room temperature prior to performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

Specimen Collection and Preparation

H. influenzae can be recovered from clinical specimens on chocolate agar. For specific recommendations, consult appropriate references.[1,5] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *H. influenzae*. After these criteria are met, serological identification can be performed.

Testing the Isolate for Autoagglutination

1. From the test culture on chocolate agar, transfer a loopful of growth to a drop of sterile 0.85% NaCl solution on a clean slide and emulsify the organism.
2. Rotate the slide for one minute and then observe for agglutination.
3. If agglutination (autoagglutination) occurs, the culture is rough and cannot be tested. Subculture to chocolate agar, incubate, and test the organism again as described in steps 1 and 2.

   If no agglutination occurs, proceed with testing the organism.

Test Procedure

Test culture isolates with Haemophilus Influenzae Poly for presumptive identification, then test with monospecific antisera.

1. Dispense 1 drop of the Haemophilus Influenzae Antiserum to be tested on an agglutination slide.
2. Transfer a loopful of growth of the test organism to the drop of antiserum and mix thoroughly.
3. Rotate the slide for one minute and read for agglutination.
4. Repeat this procedure for known positive and negative control cultures.

Results

Observe test results and record agglutination as follows:

4+ 100% agglutination; background is clear to slightly hazy.
3+ 75% agglutination; background is slightly cloudy.
2+ 50% agglutination; background is moderately cloudy.
1+ 25% agglutination; background is cloudy.
– No agglutination.

Positive control: Should produce 3+ or greater agglutination.

Negative control: Should produce no agglutination.

Positive test result: Agglutination of 3+ or greater within one minute.

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity as well as morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as *H. influenzae*.
2. Serological methods alone cannot identify the isolate as *H. influenzae*.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent a smooth suspension of the microorganism or may cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates occur and will agglutinate spontaneously causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. *H. influenzae* has antigenic similarities to several unrelated bacteria. Cross-reactions can occur between *H. influenzae* and strains of *S. pneumoniae*, *Escherichia coli* and several species of *Staphylococcus*, *Streptococcus* and *Bacillus*.
6. Haemophilus Influenzae Antisera have been tested using undiluted cultures taken from agar media. These antisera have not been tested using antigen suspensions in NaCl solution or other diluents. If the

user employs a variation of the recommended procedure, each lot of antiserum must be tested with known control cultures to verify that expected reactions are obtained under the modified procedure.

7. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
8. A rehydrated Haemophilus Influenzae Antiserum that is cloudy or develops a precipitate during use should be discarded.

References

1. **Campos, J. M.** 1995. *Haemophilus*, p. 556-565. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
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Packaging

Haemophilus Influenzae Antiserum Poly	1 ml	2237-50
Haemophilus Influenzae Antiserum Type a	1 ml	2250-50
Haemophilus Influenzae Antiserum Type b	1 ml	2236-50
Haemophilus Influenzae Antiserum Type c	1 ml	2789-50
Haemophilus Influenzae Antiserum Type d	1 ml	2790-50
Haemophilus Influenzae Antiserum Type e	1 ml	2791-50
Haemophilus Influenzae Antiserum Type f	1 ml	2792-50

Bacto® Listeria Antigens and Antisera

Listeria O Antiserum Type 1 · Listeria O Antiserum Type 4 Listeria O Antiserum Poly · Listeria O Antigen Type 1 (Slide) Listeria O Antigen Type 1 (Tube) · Listeria O Antigen Type 4 (Slide) Listeria O Antigen Type 4 (Tube)

Intended Use

Bacto Listeria O Antisera Types 1, 4, and Poly are used for identifying *Listeria monocytogenes* in the macroscopic tube and rapid slide tests.

Bacto Listeria O Antigens Types 1 and 4 (Tube) and (Slide) are used as positive controls in the macroscopic tube and rapid slide tests, respectively.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985[3] and, since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.[7] *Listeria* species are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are streptococci, enterococci, micrococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Strains of *Listeria* species are divided into serotypes based on cellular (O) and flagellar (H) antigens.[9] Thirteen serotypes of *L. monocytogenes* are known. Most human disease is caused by serotypes 1/2a, 1/2b and 4b.[10]

Principles of the Procedure

Identification of *Listeria monocytogenes* includes both biochemical and serological confirmation. Serological confirmation requires that the microorganism (antigen) react with its corresponding antibody. This in vitro reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity) and bonds strongly (high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to another species, heterologous reactions are possible. These are characterized as weak in strength or slow in formation. Such unexpected and perhaps unpredictable reactions may lead to some confusion in serological identification. A positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Agglutination of the somatic antigen in the slide test appears as a firm granular clumping. Homologous reactions occur rapidly and are strong (3+). Heterologous reactions form slowly and are weak.

The agglutination of the somatic antigen in the tube tests appears as a loose flocculation that can easily be resuspended. Homologous reactions using Listeria O Antisera should exceed a titer of 2+ at 1:320.

Reagents

Listeria O Antisera Types 1, 4, and Poly are lyophilized, polyclonal rabbit antisera containing approximately 0.04% Thimerosal as a preservative. The antisera are prepared according to procedures recommended by Gray.[11] Listeria O Antisera Types 1 and 4 are specific for the respective serotypes of *L monocytogenes* while Listeria O Antiserum Poly contains agglutinins for *L. monocytogenes* serotypes 1 and 4.

Listeria O Antigens Types 1 and 4 (Tube) and (Slide) are suspensions of appropriate *L. monocytogenes* serotypes containing 0.3% formaldehyde as a preservative. When used according to the suggested procedure, the reagents will yield the following:

REAGENT	VIAL	NUMBER OF TESTS
Listeria O Antiserum	1 ml	10 tube tests, 400 slide tests
Listeria O Antigen (Slide)	5 ml	100 slide tests
Listeria O Antigen (Tube)	25 ml	5 tube tests

User Quality Control

Identity Specifications

Listeria O Antiserum Type 1
Listeria O Antiserum Type 4
Listeria O Antiserum Poly

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

Listeria O Antigen Type 1 (Slide)
Listeria O Antigen Type 1 (Tube)
Listeria O Antigen Type 4 (Slide)
Listeria O Antigen Type 4 (Tube)

Appearance: White, liquid suspension.

Performance Response

Rehydrate Listeria O Antiserum per label directions. Perform the slide or tube agglutination test using appropriate Listeria O Antigens (Slide) or (Tube).

Slide test: An antiserum is considered satisfactory if it demonstrates a 3+ or greater reaction at 1:80 with a 1:5 dilution of the homologous antigen.

Macroscopic tube test: An antiserum is considered satisfactory if it demonstrates a 3+ or greater reaction with the 1:320 dilution of the homologous antigen.

Precautions

1. For In Vitro Diagnostic Use.
2. **Listeria O Antiserum Type 1**
   Listeria O Antiserum Type 4
   Listeria O Antiserum Poly
   The Packaging of This Product Contains Dry Natural Rubber.
3. **Listeria O Antigen Type 1 (Slide)**
   Listeria O Antigen Type 1 (Tube)
   Listeria O Antigen Type 4 (Slide)
   Listeria O Antigen Type 4 (Tube)
   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. (US) Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Kidneys, Lungs, Skin.
   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Listeria O Antisera at 2-8°C.

Store Listeria O Antigen (Slide) and (Tube) at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Listeria O Antiserum Type 1
Listeria O Antiserum Type 4
Listeria O Antiserum Poly
Listeria O Antigen Type 1 (Slide)
Listeria O Antigen Type 1 (Tube)
Listeria O Antigen Type 4 (Slide)
Listeria O Antigen Type 4 (Tube)

Materials Required But Not Provided

Rapid Slide Test
FA Buffer, Dried
Agglutination slides
Applicator sticks
Waterbath, 80-100°C
Formaldehyde
Droppers

Macroscopic Tube Test
FA Buffer, Dried
McFarland Barium Sulfate Standard No. 3
Culture tubes 12 x 75 mm and rack
Serological pipettes, 1 ml
Waterbath, 50°C
Refrigerator, 2-8°C
Formaldehyde

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Listeria O Antisera: To rehydrate, add 1 ml sterile distilled or deionized water to each vial. Rotate gently to dissolve contents completely.

Listeria O Antigens (Slide) and (Tube) are ready to use.

Specimen Collection and Preparation

From clinical specimens, *Listeria* can be recovered on selective differential media such as McBride Listeria Agar, Oxford Agar, Modified Oxford Agar, LPM Agar or Palcam Medium. For specific recommendations on isolation of *Listeria* from clinical specimens, consult appropriate references.[10,12,13] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *Listeria monocytogenes*. After these criteria are met, serological identification can be performed.

From food or dairy samples, *Listeria* can be recovered when samples are processed to recover injured microorganisms and prevent overgrowth of competing microorganisms. Consult appropriate references for recommended procedures for the isolation of *Listeria* from foods.[7,14,16] Having followed an established protocol, isolate a pure culture of the microorganism and confirm that biochemical test reactions are consistent with the identification of the organism as *Listeria monocytogenes*. After these criteria are met the serological identification can be performed.

Test Procedure

Rapid Slide Test

1. **FA Buffer, Dried**: Rehydrate per label directions.
2. **Test isolate**: Suspend growth from a solid agar medium in FA Buffer.
3. Heat the organism suspension at 80-100°C (in a waterbath) for 1 hour.
4. Centrifuge the suspension and remove the bulk of the supernatant fluid.
5. Resuspend the organism in the remaining portion of liquid.
6. **Listeria Antiserum**: On an agglutination slide, dispense 2 separate drops of the desired antiserum diluted 1:20 in NaCl solution. The first drop will be used for the test isolate and the second for the negative control.
7. **Organism suspension**: Add 1 drop of heated organism to the first drop of antiserum.
8. **Negative control**: Dispense 1 drop of FA Buffer on the agglutination slide. Add one drop of organism suspension from step 5.
9. **Positive control**: Add one drop of homologous Listeria O Antigen (Slide) to the second drop of antiserum.
10. Rotate the slide for 1-2 minutes and read for agglutination.

Slide Test Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show a 3+ or greater agglutination.
3. **Negative control**: Should show no agglutination. If agglutination occurs, the culture is rough and cannot be tested. Subculture to a non-inhibitory medium, incubate and test the organism again.
4. **Test isolates**: 3+ or greater agglutination is a positive result.
5. A partial (less than 3+) or a delayed agglutination reaction should be considered negative.

Macroscopic Tube Test

1. **Test isolate**: Suspend growth of the test organism from a solid agar medium in FA Buffer. Adjust to a density approximating that of a McFarland Barium Sulfate Standard No. 3.
2. Prepare a row of 9 culture tubes (12 x 75 mm) for each serum suspension to be tested, including the positive control.
3. **Formalized FA Buffer**: Dispense 0.9 ml formalized FA Buffer (0.3 ml formaldehyde per 300 ml FA Buffer) to the first tube in each row and 0.5 ml to the remaining tubes.
4. **Listeria O Antiserum**: Using a 1 ml serological pipette, add 0.1 ml of the desired antiserum to tube 1 in each row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. In like manner, continue transferring 0.5 ml through tube 8, discarding 0.5 ml from tube 8 after mixing. Tube 9 is an antigen control tube. Upon addition of the test suspension, final dilutions will be 1:20 through 1:2560 for tubes 1 through 8, respectively.
5. **Test Suspension**: Add 0.5 ml of the test suspension to each of 9 tubes.
6. **Positive control**: Add 0.5 ml of an appropriate Listeria O Antigen to each of 9 tubes containing antiserum.
7. **Negative control**: Add 0.5 ml of the test suspension to a tube containing FA Buffer.
8. Shake the rack to mix. Incubate in a 50°C waterbath for 2 hours. Refrigerate overnight. Read for agglutination the following morning.

Tube Test Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 2+ or greater agglutination at 1:320.
3. **Antigen control**: Tube 9 of each row should show no agglutination.

4. If results of the positive control or antigen control are not as described, the test is invalid and results cannot be read.
5. **Test serum**: The titer is that dilution which shows a 2+ or greater agglutination at 1:320.

Limitations of the Procedure

1. Serological techniques employing Listeria O Antisera serve as corroborative evidence for the identification of *Listeria monocytogenes*. Final identification cannot be made without consideration of morphological, serological and biochemical characterization.
2. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
3. Rough culture isolates occur and will agglutinate spontaneously, causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
4. Agglutination reactions of 3+ or greater in the slide test are interpreted as positive reactions. Cross-reactions resulting in a 1+ or 2+ agglutination are likely since there are somatic antigens shared among different organisms such as staphylococci, enterococci and *Bacillus* species.[10]
5. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
6. Exposure of Listeria O Antigens to temperatures below 2°C can result in autoagglutination. Antigens must be smooth uniform suspensions; examine antigen vials for agglutination before use. Suspensions with agglutination are not usable and should be discarded.
7. It is important in this test to use the recommended time and temperature of incubation. Also, care should be taken to make certain that the waterbath is in a location free of mechanical vibration.
8. Discard any Listeria O Antiserum that is cloudy or has a precipitate after rehydration or storage.

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Packaging

Listeria O Antiserum Type 1	1 ml	2300-50
Listeria O Antiserum Type 4	1 ml	2301-50
Listeria O Antiserum Poly	1 ml	2302-50
Listeria O Antigen Type 1 (Slide)	5 ml	2303-56
Listeria O Antigen Type 1 (Tube)	25 ml	2305-65
Listeria O Antigen Type 4 (Slide)	5 ml	2304-56
Listeria O Antigen Type 4 (Tube)	25 ml	2306-65
FA Buffer, Dried	6 x 10 g	2314-33
	100 g	2314-15
	10 kg	2314-08