Chapter 4
Isolation and Identification of *Shigella*

Isolation and identification of *Shigella* can be greatly enhanced when optimal laboratory media and techniques are employed. The methods presented here are intended to be economical and to offer laboratorians some flexibility in choice of protocol and media. Laboratories that do not have sufficient resources to adopt the methods described in this chapter should consider sending specimens or isolates to other laboratory facilities that routinely perform these procedures.

A. Isolation Methods

Figure 4-1 outlines the procedure for isolation of *Shigella* from fecal specimens. Refer to Annex B for a list of supplies necessary for laboratory identification of *Shigella*.

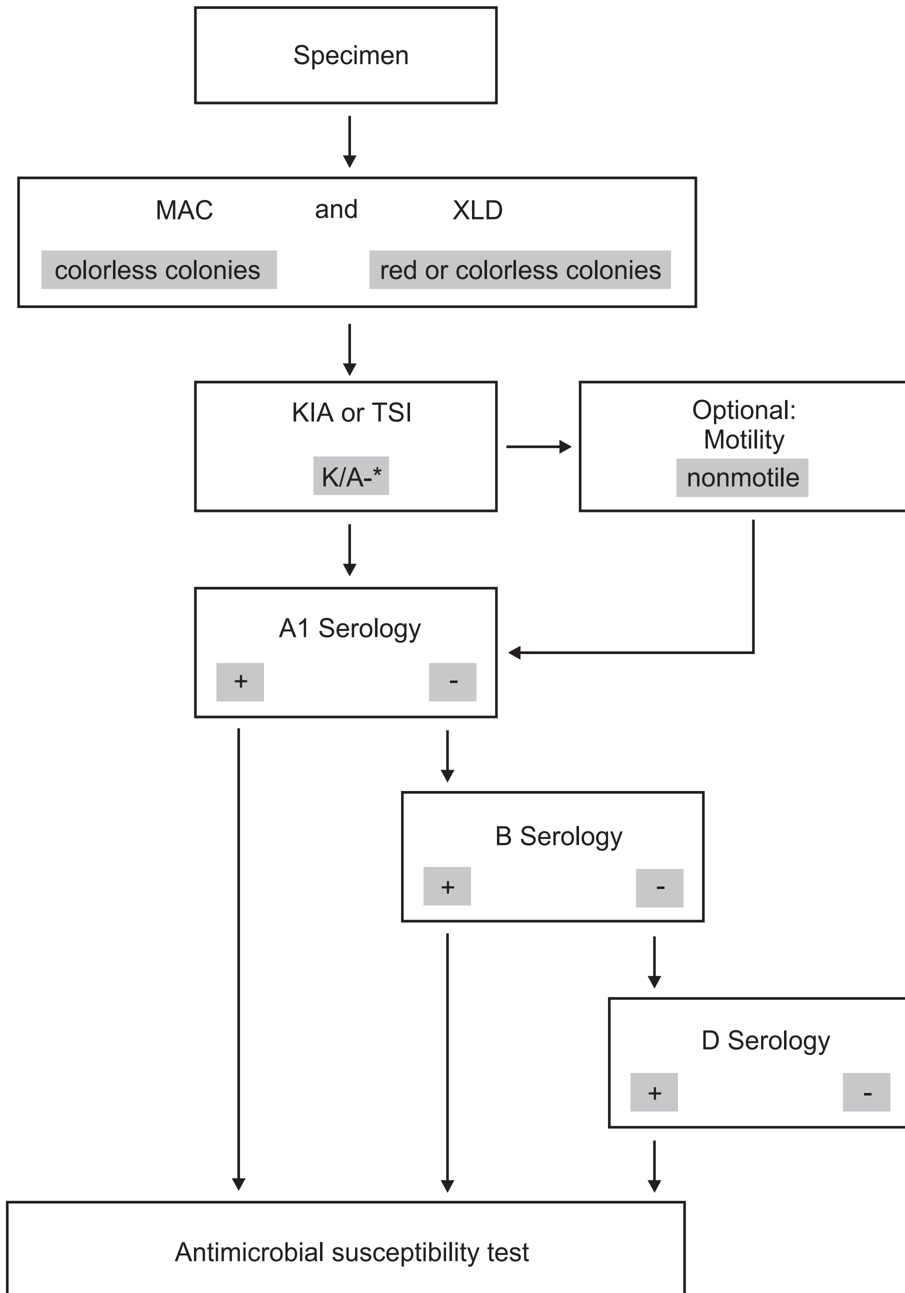
For optimal isolation of *Shigella*, two different selective media should be used: a general purpose plating medium of low selectivity, such as MacConkey agar (MAC), and a more selective agar medium, such as xylose lysine desoxycholate (XLD) agar. Desoxycholate citrate agar (DCA) and Hektoen enteric (HE) agar are suitable alternatives to XLD agar as media of moderate to high selectivity. **Do not use SS agar as it frequently inhibits the growth of *S. dysenteriae* serotype 1.**

When selective or differential media are incorrectly prepared, the reactions of organisms on those media can be affected. Therefore, it would be helpful to refer to Section D, "Media for isolation and identification of *Shigella*," for a discussion of these media, their preparation, and appropriate quality control strains.

There is no enrichment medium for *Shigella* that consistently provides a greater recovery rate than use of direct plating alone.

1. Inoculation of selective agar

Fecal specimens should be plated as soon as possible after arrival in the laboratory. Selective media may be inoculated with a single drop of liquid stool or fecal suspension. Alternatively, a rectal swab or a fecal swab may be used. If a swab is used to inoculate selective media, an area approximately 2.5 cm (1 inch) in diameter is seeded on the agar plates, after which the plates are streaked for isolation (Figure 4-2). Media of high selectivity such as XLD require more overlapping when streaking than media of low selectivity. When inoculating specimens to a plate for isolation, it is important to use the entire plate to increase the chances of obtaining well-isolated colonies. Incubate the plates for 18 to 24 hours at 35° to 37°C.



*K = alkaline (red); A = acid (yellow); - = no H_2S produced

Figure 4-1. Procedure for recovery of *Shigella* from fecal specimens

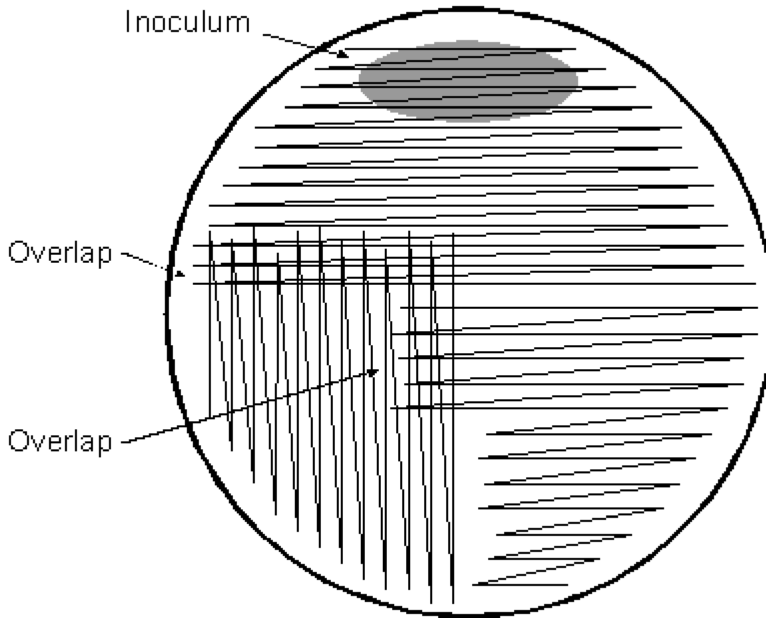


Figure 4-2. Method of streaking plating medium for isolation of *Shigella*

2. Isolation of suspected *Shigella*

After incubation, record the amount and type of growth (e.g., lactose-fermenting or lactose-nonfermenting) on each isolation medium for each patient specimen (a sample worksheet is presented in Figure 4-3). Colonies of *Shigella* on MAC appear as convex, colorless colonies about 2 to 3 mm in diameter. *S. dysenteriae* 1 colonies may be smaller (Table 4-1). *Shigella* colonies on XLD agar are transparent pink or red smooth colonies 1 to 2 mm in diameter. *S. dysenteriae* 1 colonies on XLD agar are frequently very tiny, unlike other *Shigella* species. Figures 4-4 to 4-7 show the typical appearance of *Shigella* on XLD and MAC. Select suspect colonies from the MAC and XLD plates and inoculate to appropriate screening media such as Kligler iron agar (KIA) or triple sugar iron agar (TSI).

Table 4-1. Appearance of *Shigella* colonies on selective plating media

Selective agar medium	Color of colonies	Size of colonies
MAC	Colorless	2-3 mm[a,b]
XLD	Red or colorless	1-2 mm[a,c]
DCA	Colorless	2-3 mm[a]
HE	Green	2-3 mm[a]

[a] *S. dysenteriae* 1 colonies may be smaller.
[b] See Section D for discussion of different formulations of commercial dehydrated MacConkey agar and how selectivity is affected for isolation of *Shigella.*
[c] *S. dysenteriae* 1 colonies on XLD agar are frequently very tiny, unlike other *Shigella* species.

B. Biochemical Screening Tests

Identification of *Shigella* spp. involves both biochemical and serologic testing. The use of biochemical screening media is usually advisable to avoid wasting antisera. Most laboratories will find KIA (or TSI) to be the single most helpful medium for screening suspected *Shigella* isolates. If an additional test is desired, motility medium can be used to screen isolates before doing serologic testing. Section D in this chapter further describes these media.

1. Kligler iron agar and triple sugar iron agar

To obtain true reactions in KIA or TSI or other biochemical tests, it is necessary to inoculate with a pure culture. Carefully select at least one of each type of well-isolated colony on each plate. Using an inoculating needle, lightly touch only the very center of the colony. Do not take the whole colony or go through the colony and touch the surface of the plate. This is to avoid picking up contaminants that may be on the surface of the agar. If there is doubt that a particular colony is sufficiently isolated from surrounding colonies, purify the suspicious colony by streaking on another agar plate, after which the KIA or TSI slant may be inoculated.

KIA and TSI are inoculated by stabbing the butt and streaking the surface of the slant. After incubation for 18 to 24 hours at 35° to 37°C, the slants are observed for reactions typical of *Shigella*. When incubating most biochemicals, caps should be loosened before placement in the incubator. This is particularly important for KIA and TSI. If the caps are too tight and anaerobic conditions exist, the characteristic reactions of *Shigella* spp. may not occur and a misleading result could be exhibited. It is also important that KIA and TSI be prepared so that the tubes have a deep butt and a long slant (see Section D).

Shigella characteristically produces an alkaline (red) slant and an acid (yellow) butt, little or no gas, and no H_2S (Table 4-2; Figure 4-8). A few strains of *S. flexneri* serotype 6 and very rare strains of *S. boydii* produce gas in KIA or TSI.

Shigella Worksheet

						OPTIONAL				SLIDE SEROLOGY			
SPECIMEN NUMBER	MEDIA	XYL/ LAC- [a]	XYL/ LAC+ [b]	COLONY	KIA/TSI	MOT [c]	UREA	LIA		A1	B	D	IDENTIFICATION
	XLD			X1									
				X2									
				X3									
	MAC			M1									
				M2									
				M3									
	XLD			X1									
				X2									
				X3									
	MAC			M1									
				M2									
				M3									
	XLD			X1									
				X2									
				X3									
	MAC			M1									
				M2									
				M3									

[a]XYL/LAC - = Xylose or lactose negative colonies [b]XYL/LAC + = Xylose or lactose positive colonies [c]MOT = Motility

Figure 4-3. *Shigella* worksheet

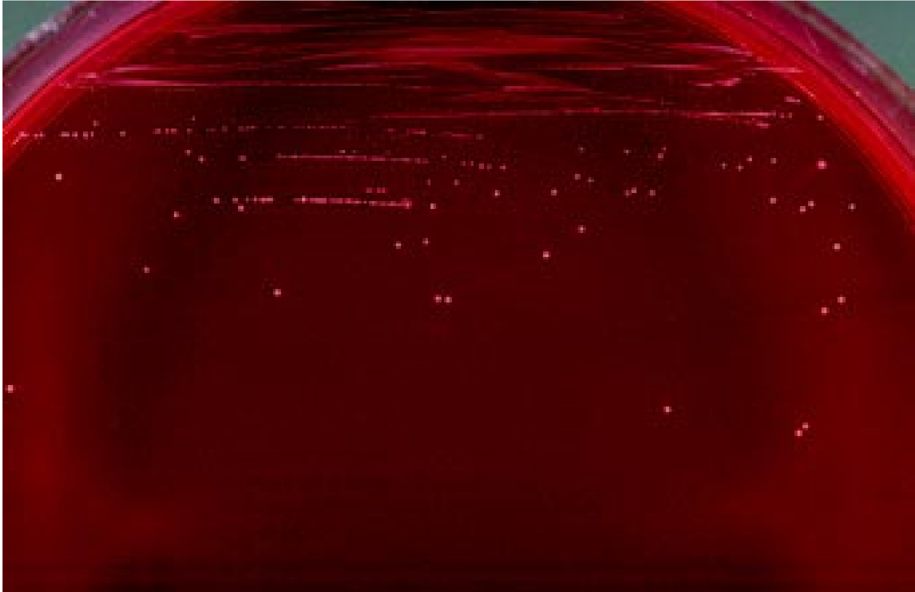


Figure 4-4. *S. dysenteriae* 1 colonies on XLD

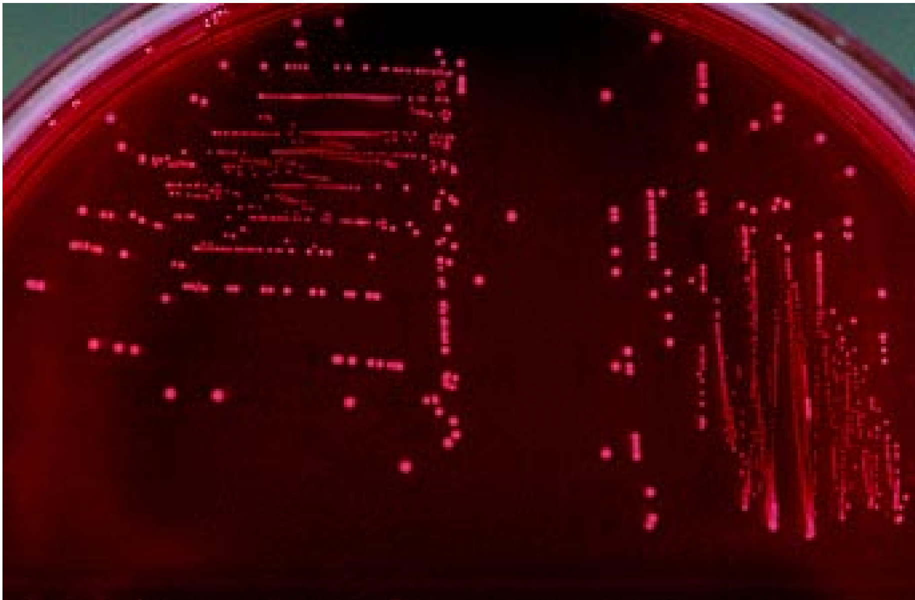


Figure 4-5. *S. flexneri* colonies on XLD

Figure 4-6. *S. flexneri* and *E. coli* colonies on XLD. *S. flexneri* colonies are colorless to red while the *E. coli* colonies are yellow.

Figure 4-7. *S. flexneri* colonies appear colorless on MAC. *E. coli* colonies are pink to red.

Table 4-2. Reactions of *Shigella* in screening biochemicals

Screening medium	*Shigella* reaction
KIA	K/A, no gas produced (red slant/yellow butt)[a]
TSI	K/A, no gas produced (red slant/yellow butt)[a]
H_2S (on KIA or TSI)	Negative
Motility	Negative
Urea	Negative
Indole	Positive or negative
LIA	K/A (purple slant/yellow butt)[b]

[a] K = alkaline (red); A = acid (yellow); some strains of *S. flexneri* serotype 6 and *S. boydii* produce gas from glucose.

[b] K = alkaline (purple); A = acid (yellow); an alkaline reaction (purple) in the butt of the medium indicates that lysine was decarboxylated. An acid reaction (yellow) in the butt of the medium indicates that lysine was not decarboxylated.

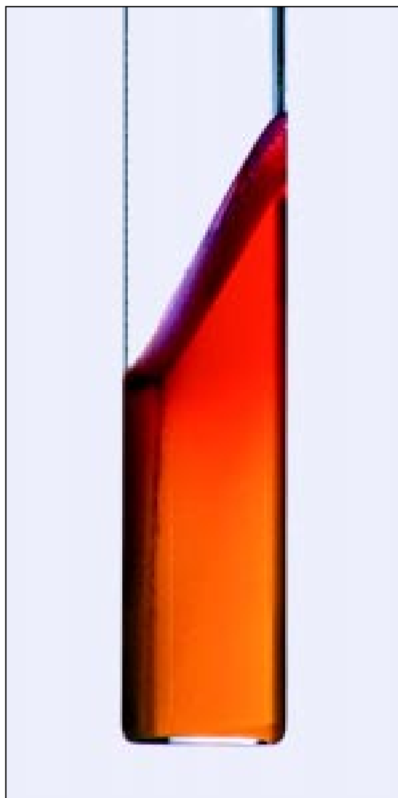


Figure 4-8. Reaction typical of *Shigella* in KIA (alkaline slant and acid butt)

2. Motility agar

Motility agar should be inoculated with a straight inoculating needle, making a single stab about 1 to 2 cm down into the medium. Motility agar may be inoculated with growth from a KIA or TSI that shows a reaction typical of *Shigella*. Alternately, motility agar can be inoculated at the same time as the KIA or TSI slant by using the same inoculating needle without touching the colony again. The motility agar should be inoculated first, after which, the KIA or TSI is inoculated by stabbing the butt first and then streaking the surface of the slant. Do not select a second colony to inoculate the KIA or TSI after the motility agar has been inoculated since it may represent a different organism.

Examine after overnight incubation at 35° to 37°C. Motility is indicated by the presence of diffuse growth (appearing as clouding of the medium) away from the line of inoculation (Figure 4-9). Nonmotile organisms do not grow out from the line of inoculation. Motility reactions may be difficult for inexperienced laboratorians to read; therefore reactions should be compared with positive and negative control strains. *Shigella* spp. are always nonmotile (Table 4-2).

The surface of the motility agar should be dry when used. Moisture can cause a nonmotile organism to grow down the sides of the agar creating a haze of growth and appearing to be motile (see Section D).

Sulfide-indole-motility medium is a combination medium that is commercially available in dehydrated form (see Section D). It can be used in place of motility medium.

Figure 4-9. Motility medium showing a nonmotile organism in the left tube and a motile organism in the right tube

3. Additional biochemical screening tests

Other biochemical tests such as urea medium and lysine iron agar may be used for additional screening of isolates before testing with antisera. The value of these should be assessed before using them routinely (Table 4-2, Annex G). These media, their preparation, and suggested quality control strains are described in Section D.

Urea medium

Urea medium screens out urease-producing organisms such as *Klebsiella* and *Proteus*. Urea agar is inoculated heavily over the entire surface of the slant. Loosen caps before incubating overnight at 35° to 37°C. Urease positive cultures produce an alkaline reaction in the medium, evidenced by a pinkish-red color (Figure 4-10). Urease negative organisms do not change the color of the medium, which is a pale yellowish-pink. *Shigella* spp. are always urease negative (Table 4-2).

Lysine iron agar

Lysine iron agar (LIA) is helpful for screening out *Hafnia* spp. and certain *E. coli*, *Proteus*, and *Providencia* strains. LIA should be inoculated by stabbing the butt and streaking the slant. After incubation for 18 to 24 hours at 35° to 37°C, organisms that produce lysine decarboxylase in LIA cause an alkaline reaction (purple color) in the butt of the medium and also on the slant (Figure 4-11). H_2S production is indicated by a blackening of the medium. Organisms lacking lysine decarboxylase, produce an alkaline slant (purple) and an acid butt (yellow), no gas, and no H_2S. *Proteus* and *Providencia* spp. will often produce a red slant caused by deamination of the lysine. LIA must be prepared so that the tubes have a deep butt (see Section D).

Shigella spp. are lysine negative and characteristically produce an alkaline (purple) slant and an acid (yellow) butt, no gas, and no H_2S (Table 4-2).

C. Serologic Identification of *Shigella*

Serologic testing is needed for the identification of *Shigella* isolates. The genus *Shigella* is divided into four serogroups, each group consisting of a species that contains distinctive type antigens. The serogroups A, B, C, and D correspond to *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, respectively. Three of the four, *S. dysenteriae*, *S. flexneri*, and *S. boydii,* are made up of a number of serotypes (see Chapter 3, Table 3-1).

Serologic identification is performed typically by slide agglutination with polyvalent somatic (O) antigen grouping sera, followed, in some cases, by testing with monovalent antisera for specific serotype identification. Monovalent antiserum to *S. dysenteriae* 1 is required to identify this serotype, which is the most frequent cause of severe epidemic dysentery. Once one colony from a plate has been identified as *Shigella*, no further colonies from the same specimen need to be tested.

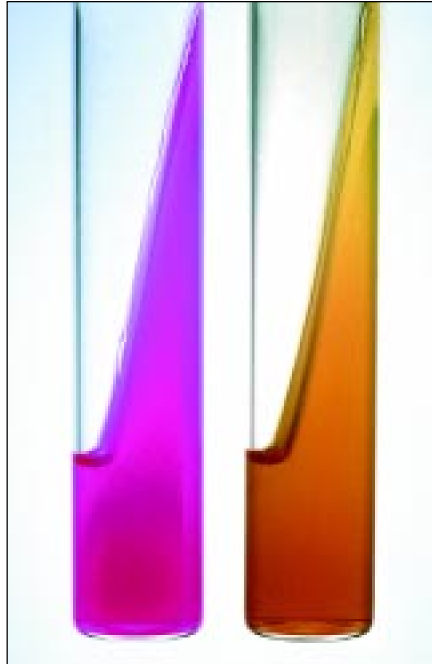


Figure 4-10. A pink color develops in a positive urease reaction (tube on left)

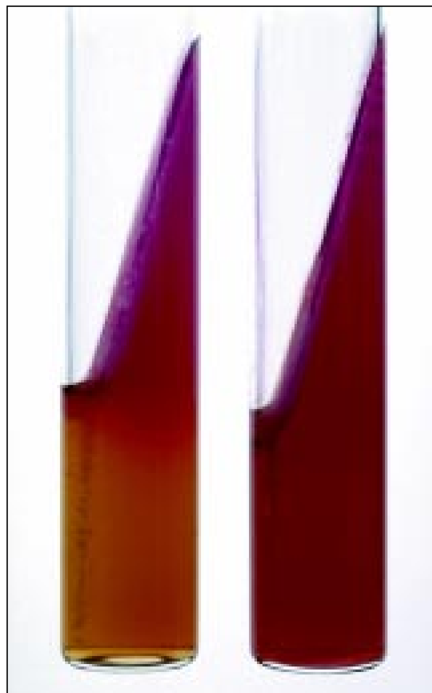


Figure 4-11. Organisms positive for lysine decarboxylase produce a purple color throughout the LIA medium (tube on right). Lysine-negative organisms produce a yellow color (acid) in the butt portion of the tube (tube on left).

Laboratorians should be aware that some *Shigella* commercial antiserum is labeled or packaged differently; for example, *Shigella* polyvalent A, which includes antisera to serotypes 1 through 7, may also be labeled polyvalent A1. Also, monovalent antiserum may be labeled in a way that it may be confused with polyvalent antiserum; for example, monovalent antiserum to *S. dysenteriae* 1 may be labeled "*Shigella* A1" instead of "*S. dysenteriae* serotype 1". When using newly purchased antisera, the laboratorian should read the package insert or check with the manufacturer if the label is not self-explanatory.

1. Slide agglutination

Because *S. dysenteriae* 1 (followed by *S. flexneri* and *S. sonnei*) is the most common agent of epidemic dysentery, isolates that react typically in the screening biochemicals should be screened first with monovalent A1 antiserum, then with polyvalent B antiserum, and finally in polyvalent D antiserum.

Agglutination tests may be carried out in a petri dish or on a clean glass slide. An inoculating loop or needle, sterile applicator stick or toothpick is used to remove a portion of the growth from the surface of KIA, TSI, heart infusion agar (HIA), or other nonselective agar medium. Serologic testing should not be done on growth from selective media such as MAC or XLD because this may give false-negative results. Emulsify the growth in two small drops of physiological saline and mix thoroughly. Add a small drop of antiserum to one of the suspensions. Usually approximately equal volumes of antiserum and growth suspension are mixed, but the volume of suspension may be as much as double the volume of the antiserum. To conserve antiserum, volumes as small as 10 microliters can be used. An inoculating loop may be used to dispense small amounts of antisera if micropipettors are not available (Figure 4-12). Mix the suspension and antiserum well and then tilt the slide back and forth to observe for agglutination. If the reaction is positive, clumping will appear within 30 seconds to 1 minute (Figure 4-13). Examine the saline suspension carefully to ensure that it is even and does not show clumping due to autoagglutination. If autoagglutination occurs, the culture is termed "rough" and cannot be serotyped.

Cultures that react serologically and show no conflicting results in the biochemical screening tests are reported as positive for *Shigella*. Serologically negative isolates that are biochemically identified as *Shigella* may be sent to a reference laboratory.

2. Quality control of antisera

All lots of antisera should be quality controlled before use. Quality control of antisera is discussed in Chapter 11.

D. Media for Isolation and Identification of *Shigella*

This section contains descriptions of all media mentioned in this chapter and discussions of their characteristics, preparation, and appropriate quality control strains. Each manufacturer's lot number of commercial dehydrated media or each

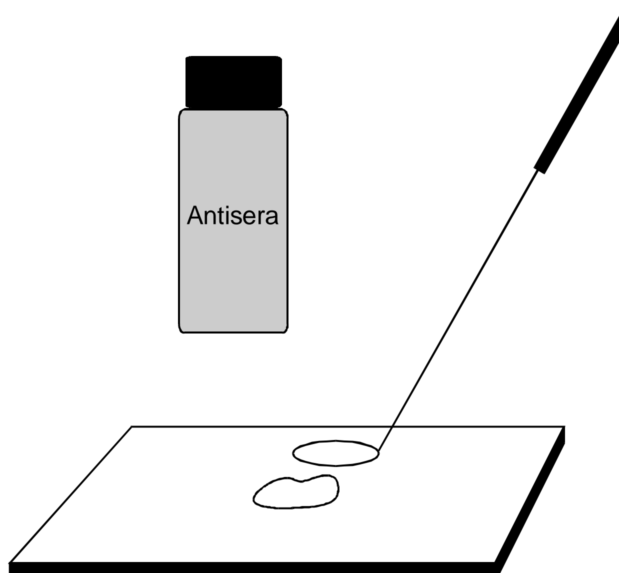


Figure 4-12. A bent loop may be helpful in dispensing small amounts of antiserum for slide agglutination tests.

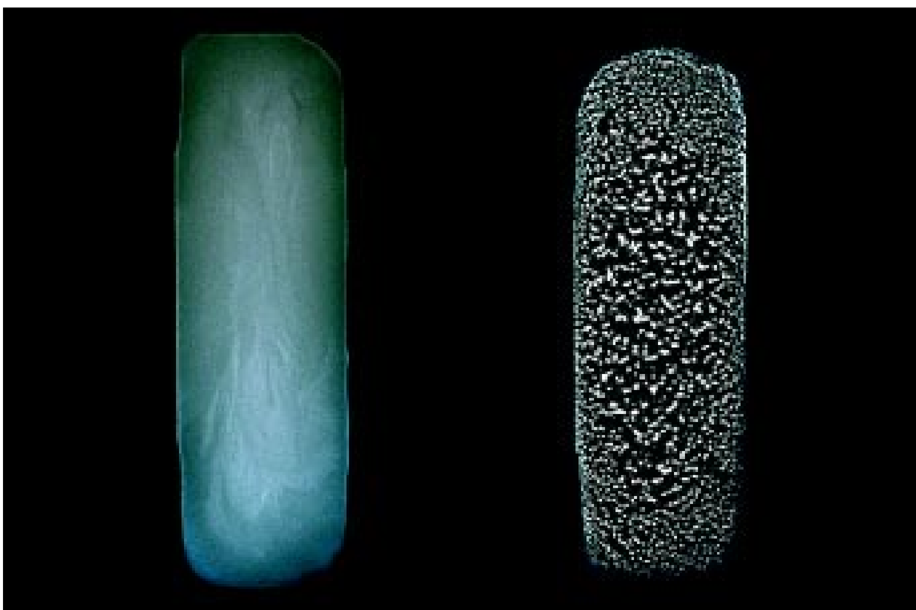


Figure 4-13. *Shigella* antiserum will agglutinate strains of the same serogroup or serotype (right). *Shigella* will not agglutinate when mixed with saline (left).

batch of media prepared from individual ingredients should be quality controlled before use. See Chapter 11 for a description of appropriate quality control methods.

1. Desoxycholate citrate agar

Desoxycholate citrate agar (DCA) is a differential selective plating medium for the isolation of enteric pathogens, particularly *Shigella* and *Salmonella*. Lactose-fermenting organisms produce pink colonies surrounded by a zone of bile precipitation. Colonies of lactose-nonfermenting strains are colorless. Several formulations of DCA, which may vary in selectivity, are available from different manufacturers.

Preparation and quality control

Prepare according to manufacturer's instructions. [Note: It may also be prepared from individual ingredients, but this can result in much greater lot-to-lot variation than when prepared from commercial dehydrated preparations.] DCA medium is very heat-sensitive, and overheating during boiling should be avoided. Do not autoclave. Plates can be stored at 4°C for up to a week.

For quality control of DCA, the following organisms should be adequate for confirmation of selective and inhibitory growth characteristics: *E. coli* may be somewhat inhibited, depending on the particular formulation used, but will produce pink colonies surrounded by a zone of precipitated bile; *S. flexneri* and *S. dysenteriae* 1 will produce fair to good growth of colorless colonies.

2. Hektoen enteric agar

Hektoen enteric agar (HE) is a differential selective agar that is useful for isolation of *Salmonella* and *Shigella.* It has an H_2S-indicator system for selecting H_2S-producing *Salmonella*, which produce blue-green colonies with a black center. *Shigella* colonies are green while rapid lactose-fermenters such as *E. coli* are pink to orange with a zone of bile precipitation.

Preparation and quality control

Prepare according to manufacturer's instructions. [Note: Several commercial brands of HE are available. This medium can also be prepared from individual ingredients, but results may be much more variable than with a commercial dehydrated formulation.] Heat to boiling to dissolve, but avoid overheating. Do not autoclave. When cool enough to pour, dispense into plates. Plates can be stored at 4°C for up to 1 week.

For quality control of HE, the following organisms should be adequate for confirmation of selective and inhibitory growth characteristics: *E. coli* should produce colonies that are pink to orange surrounded by a bile precipitate; *S. flexneri* should produce fair to good growth of green colonies, but *S. dysenteriae* 1 colonies should be smaller.

3. Kligler iron agar and triple sugar iron agar

Kligler iron agar (KIA) and triple sugar iron (TSI) agar are carbohydrate-containing screening media widely used for identification of enteric pathogens, including *Shigella*. Both media differentiate lactose fermenters from nonfermenters and have a hydrogen sulfide indicator. H_2S-producing organisms will cause blackening of the medium in both KIA and TSI.

KIA contains glucose and lactose. Organisms which ferment glucose cause the butt of the tube to become acid (yellow); some also produce gas. Lactose-fermenting organisms will produce an acid (yellow) slant; lactose-nonfermenting organisms will have an alkaline (red) slant.

TSI contains sucrose in addition to the ingredients in KIA. Organisms which ferment either lactose or sucrose will produce an acid (yellow) slant while organisms that ferment neither carbohydrate will have an alkaline (red) slant. As in KIA, glucose-fermenters produce an acid (yellow) reaction in the butt (sometimes with gas produced).

Preparation and quality control

Prepare according to manufacturer's instructions. [Note: There are several commercially available dehydrated formulations of KIA and TSI. These media can also be prepared from individual ingredients, but there may be lot-to-lot variation.] Dispense a quantity of medium in appropriate containers such that the volume of medium is sufficient to give a deep butt and a long slant. For example, dispense 6.5 ml of medium into 16 x 125-mm screw-cap tubes (leave caps loose), and after autoclaving allow the slants to solidify in a manner such that the medium in the butt of the tube is about 3.5 cm deep and the slant is about 2.5 cm long. Tighten caps and store at 4°C for up to 6 months.

For quality control of KIA or TSI, the following organisms should be adequate for confirmation of biochemical response characteristics: *E. coli* should give an acid slant and butt, with the production of gas but no H_2S; *S. flexneri* should give an alkaline slant, acid butt, without production of gas or H_2S (Figure 4-8); an H_2S-producing *Salmonella* may be used to control this reaction.

4. Lysine iron agar

Organisms that produce lysine decarboxylase in LIA cause an alkaline reaction (purple color) in the butt of the medium and also on the slant (Figure 4-11). H_2S production is indicated by a blackening of the medium. Organisms lacking lysine decarboxylase, such as *Shigella*, typically produce an alkaline slant (purple) and an acid butt (yellow) no gas, and no H_2S (Table 4-2). *Proteus* and *Providencia* spp. will often produce a red slant caused by deamination of the lysine. LIA must be prepared so that the volume of medium in the tube is sufficient to give a deep butt. It is important for LIA tubes to have a deep butt because the decarboxylation reaction occurs only in anaerobic conditions.

Preparation and quality control

Prepare medium according manufacturer's instructions on the bottle. [Note: Several companies sell dehydrated LIA. LIA may also be prepared from individual ingredients, but there may be lot-to-lot variation.] Dispense a quantity of medium in appropriate containers such that the volume of medium is sufficient to give a deep butt and a long slant. For example, dispense 6.5 ml of medium into 16 x 125-mm screw-cap tubes (leave caps loose), and after autoclaving allow the slants to solidify in a manner such that the medium in the butt of the tube is 3.5 cm deep and the slant is 2.5 cm long. Tighten caps and store at 4°C for up to 6 months.

For quality control of LIA, the following organisms may be used: *S. flexneri* should produce an alkaline slant and an acid butt without production of H_2S; an H_2S-producing *Salmonella* strain may be used to control the H_2S reaction and will most likely be lysine-positive and give an alkaline reaction in the butt of the tube.

5. MacConkey agar

MacConkey agar (MAC) is a differential plating medium recommended for use in the isolation and differentiation of lactose-nonfermenting, gram-negative enteric bacteria from lactose-fermenting organisms. Colonies of *Shigella* on MAC appear as convex, colorless colonies about 2 to 3 mm in diameter. *S. dysenteriae* 1 colonies may be smaller.

Several commercial brands of MAC are available. Most manufacturers prepare several formulations of MAC, which may vary in selectivity and thereby affect the isolation of *Shigella*. For example, some formulations of MAC do not contain crystal violet, a selective agent; these types are not as selective and should not be used for isolation of *Shigella*. Oxoid MacConkey Agar No. 3, Difco Bacto MacConkey Agar, and BBL MacConkey Agar are all suitable.

Preparation and quality control

Prepare according to manufacturer's instructions. [Note: MAC can also be prepared from individual ingredients, but this produces much more variable results than a commercial dehydrated formulation.] Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour into petri plates. Leave lids ajar for about 20 minutes so that the surface of the agar will dry. Close lids and store at 4°C for up to 1 month. If plates are to be stored for more than a few days, put them in a sealed plastic bag to prevent drying.

For quality control of MAC, the following organisms should be adequate for confirmation of selective and inhibitory growth characteristics: *E. coli* should produce pink to red colonies with good to excellent growth; *S. flexneri* should produce colorless colonies with fair to good growth, but *S. dysenteriae* 1 colonies may be smaller.

6. Motility medium

Because *Shigella* spp. are always nonmotile, motility medium is a useful biochemical screening test. Motility is indicated by the presence of diffuse growth (appearing as clouding of the medium) away from the line of inoculation (Figure 4-9). Nonmotile organisms do not grow out from the line of inoculation.

Preparation and quality control

Follow manufacturer's instructions to weigh out and suspend dehydrated medium. [Note: Several commercial dehydrated formulations of motility agar are available. This medium can also be prepared from individual ingredients.] Heat to boiling to make sure medium is completely dissolved. Dispense into tubes or other types of containers, leaving caps loose, and sterilize at 121°C for 15 min. Allow to solidify upright, forming a deep butt with no slant (e.g., about 4 to 5 ml of medium per 13 x 100-mm screw-cap tube). When the medium is solidified and cooled, leave caps loose until the surface of the medium has dried. Tighten caps and store at 4°C for up to 6 months.

For quality control of motility medium, the following organisms may be used: *E. coli* is motile, while *Shigella* spp. are nonmotile. The surface of the medium should be dry when used. If moisture has accumulated in the tube, carefully pour it out before inoculating the tube. Moisture can cause a nonmotile organism to grow down the sides of the agar creating a haze of growth and appearing to be motile.

7. Sulfide-indole-motility medium

Sulfide-indole-motility medium (SIM) is a commercially available combination medium that combines three tests in a single tube: hydrogen sulfide (H_2S) production, indole production, and motility. The indole reaction is not useful for screening suspected *Shigella* isolates because strains vary in their reactions in this test. It is inoculated in the same way as motility agar, by using a needle to stab about 1 to 2 cm down into the medium, and is incubated overnight at 35° to 37°C. The motility reaction is read the same as for motility medium. As in KIA or TSI, H_2S production is indicated by blackening of the medium. Indole production can be tested by either the filter paper method or by adding Kovac's reagent to the tube.

Preparation and quality control

Follow manufacturer's instructions to weigh out and suspend dehydrated medium. Heat to boiling to make sure the medium is completely dissolved. Dispense into tubes and sterilize by autoclaving for 15 minutes at 121°C.

For quality control of SIM medium, the following organisms may be used: *E. coli* is indole positive, H_2S negative, and motility positive; an H_2S-producing *Salmonella* strain may be used to control the H_2S reaction and will most likely be

motile and indole negative; *Shigella* spp. are motility negative and H_2S negative but are variable for the indole reaction.

8. Urea medium

Urease-positive cultures produce an alkaline reaction in the medium, evidenced by a pinkish-red color (Figure 4-10). Urease-negative organisms do not change the color of the medium, which is a pale yellowish-pink. *Shigella* spp. are always urease-negative (Table 4-2).

Preparation and quality control

Follow manufacturer's instructions for preparation. [Note: Several commercial brands of urea medium are available, some of which require the preparation of a sterile broth which is added to an autoclaved agar base. Some manufacturers have sterile prepared urea concentrate available for purchase.]

Prepare urea agar base as directed on the bottle. Sterilize at 121°C for 15 min. Cool to 50° to 55°C, then add urea concentrate according to manufacturer's directions. Before adding the urea to the agar base, make sure the agar base is cool since the urea is heat labile. Mix and distribute in sterile tubes. Slant the medium so that a deep butt is formed.

For quality control of urea medium, the following organisms may be used: *Proteus* spp. produce urease; *E. coli* is urease negative.

9. Xylose lysine desoxycholate agar

Xylose lysine desoxycholate agar (XLD) is a selective differential medium suitable for isolation of *Shigella* and *Salmonella* from stool specimens. Differentiation of these two species from nonpathogenic bacteria is accomplished by xylose and lactose fermentation, lysine decarboxylation, and hydrogen sulfide production.

Shigella colonies on XLD agar are transparent pink or red smooth colonies 1 to 2 mm in diameter (Figure 4-5). *S. dysenteriae* 1 colonies on XLD agar are frequently very tiny, unlike other *Shigella* species (Figure 4-4). Coliforms appear yellow (4-6). *Salmonella* colonies are usually red with black centers but may be yellow with black centers.

Preparation and quality control

Prepare according to manufacturer's instructions. [Note: Several commercial brands of XLD agar are available. This medium can also be prepared from individual ingredients, but results may be much more variable than with a commercial dehydrated formulation.] Mix thoroughly. Heat with agitation just until the medium boils. **Do not overheat;** overheating when boiling XLD or allowing the medium to cool too long may cause the medium to precipitate. Cool flask under running water until just cool enough to pour. Avoid cooling the medium too long. Pour into petri plates, leaving the lids ajar for about 20 minutes so that the

surface of the agar will dry. Plates can be stored at 4°C for up to a week.

For quality control of XLD, the following organisms should be adequate for confirmation of selective and inhibitory growth characteristics: *S. flexneri* should produce fair to good growth of transparent pink or red smooth colonies that are 1 to 2 mm in diameter; *S. dysenteriae* 1 may produce very small transparent or red colonies; *E. coli* should produce poor to fair growth of yellow colonies.

References

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