

National Veterinary Services Laboratories	
Document Title: Phage Typing of <i>Salmonella enteritidis</i>	
Author/Position: Brenda Morningstar, Microbiologist	Document Number: SOP-BTYP-1100.03
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Ward L R, DeSa J D H, and Rowe B. A phage-typing scheme for *Salmonella enteritidis*. *Epidemiology and Infection* 99. pp 291-294.
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Approved: /s/ Matthew Erdman

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1. Introduction

The Bacterial Typing Team of the Diagnostic Bacteriology Laboratory began phage typing *Salmonella enteritidis* isolates in 1990. This test was added because of the dramatic increase of *S. enteritidis* in Europe and the U.S.A. in poultry and its association with human illness. Phage type 4 was of particular interest because it was the prevalent phage type associated with public health problems in Europe. The method used is that used by the Central Public Health Laboratory, London.

2. Equipment and supplies

- 2.1 Incubator, 37-41 C
- 2.2 Incubator, 35-36 C
- 2.3 Pasteur pipettes, 5 3/4 in.
- 2.4 Micropipettor, 1-20 µl range
- 2.5 Micropipettor, 20-200 µl range
- 2.6 Disposable 10 ml pipettes, sterile
- 2.7 Sterile disposable micropipet tips, 200 µl size
- 2.8 Phage typing apparatus, LEEC, UK
- 2.9 Phage trays
- 2.10 Pipette bulb, 1 ml size
- 2.11 Magnifying lens, 10X

3. Reagents and media

- 3.1 Nutrient Agar Plates, National Veterinary Services Laboratories (NVSL) medium number 10530
- 3.2 Nutrient Broth, NVSL medium number 10221
- 3.3 Set of 16 phages, Central Public Health Laboratory, London, United Kingdom
- 3.4 Set of *S. enteritidis* type strains

4. Performance of the test

- 4.1 Isolates to be phage typed have been serotyped as *S. enteritidis*.
- 4.2 Dry nutrient agar plates by placing open plates in 37-41 C incubator for 2 hr.
- 4.3 Transfer *S. enteritidis* isolate to a tube of nutrient broth and incubate about 2-3 hr at 37-41 C until growth is visible.
- 4.4 Transfer *S. enteritidis* type strains phage type 1 and 8 as in 4.3 to be used as controls, once each week.
- 4.5 Prepare the phage tray by diluting the phages as specified on each vial of concentrated phage. Use a micropipettor with sterile tips to add enough phage to a tube containing 5 ml of nutrient broth to achieve a working dilution as specified. Use sterile Pasteur pipettes to fill wells in phage tray in correct order (written on bottom of phage tray).
- 4.6 Use a sterile, 5 3/4 in. Pasteur pipette to flood nutrient agar plate with nutrient broth. Be sure to cover entire surface of plate with broth and remove excess with pipette. Use a separate plate for each sample to be tested and each control.
- 4.7 Prepare phage typing machine for use by filling the stainless steel tray with alcohol and lighting the flame jets.
- 4.8 Use the phage typing machine to place drops of the diluted phage on the flooded nutrient agar plates. The loops of the machine are dipped in alcohol and burned off after phage is applied to each plate.
- 4.9 Allow the drops of phage to dry for a few minutes, invert the plates, and incubate at 35-36 C overnight.
- 4.10 After overnight incubation, remove the plates from the incubator and examine the plates for lysis, recording results on a phage typing worksheet.

5. Interpretation of test results

5.1 Results are interpreted as patterns of lysis as noted in Table 1 of "A Phage-typing Scheme for *Salmonella Enteritidis*" by L.R. Ward, et al. No lysis is -; 1-20 plaques is +; 21-80 plaques is ++; 81-100 plaques is +++; SCL is semiconfluent lysis; CL is confluent clear lysis; and OL is confluent opaque lysis.

5.2 Each phage type has its own pattern of lysis by the 16 typing phages as shown in Table 1. For example, phage type 8 is lysed by phages 3-10, while phage type 13 is only lysed by phages 4, 6, and 9.

5.3 The results of the two control plates are used to ensure the typing phages are working correctly.

6. Reporting of test results

6.1 The phage type is noted on the worksheet, then written on the 10-3 form in red ink next to the serotype code (column 18).

6.2 The report and worksheet are checked for accuracy by a laboratory worker.

6.3 The 10-3 form is given to the secretary who enters the results in the computer. The report is checked by the microbiologist or head of the section who validates the completed report. After the report has been validated, it is either FAXed or mailed to the submitter.

7. References

7.1 Ward L R, DeSa J D H, and Rowe B. A phage-typing scheme for *Salmonella enteritidis*. *Epidemiology and Infection* 99. pp 291-294.

8. Summary of revisions

Version 3

The format was updated and author's name changed.

National Veterinary Services Laboratories	
Document Title: <i>Salmonella</i> Serotyping—Determination of O Antigen	
Author/Position: Brenda Morningstar-Shaw, Microbiologist	Document Number: BTYPSOP1102.05
Effective Date: October 9, 2008	Supersedes: BTYPSOP1102.04

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**NVSLQSOP0006
BTYPFMA1102**

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Approved: /s/ Matt Erdman

Date: 10/1/2008

National Veterinary Services Laboratories	
Document Title: <i>Salmonella</i> Serotyping—Determination of O Antigen	
Author/Position: Brenda Morningstar-Shaw, Microbiologist	Document Number: BTYPSOP1102.05
	Supersedes: BTYPSOP1102.04

1. Purpose/Scope

Salmonella isolates have been logged in, sub cultured, and a live O antigen has been prepared (see the current version of **BTYPSOP1101**). The next step in the serotyping procedure is the determination of the O (somatic or cell wall) antigen.

2. Definitions

Not applicable

3. Safety Precautions

Salmonella is a potential human pathogen. Gloves and laboratory clothing must be worn when working with live *Salmonella* cultures. All personnel are trained according to **NVSLQOSOP0006** (Training NVSL Employees).

4. Equipment and Materials Required

- 4.1 Saline suspension of cells from blood agar base slants (BAB), NVSL medium 10008 (see the current version of **BTYPSOP1101**)
- 4.2 Glass slides or glass plate with etched 1 inch squares
- 4.3 *Salmonella* O antisera, rabbit origin, working dilution
- 4.4 0.85% Saline with 0.5% phenol, NVSL medium 30092
- 4.5 Sterile Pasteur pipettes, 5¾ inch
- 4.6 Pipette bulb, 1 ml
- 4.7 Water bath, 48±2 C
- 4.8 Ethyl alcohol, 95%
- 4.9 Disposable inoculating loop or wire
- 4.10 Acriflavin, 1:500 solution, NVSL medium 30002
- 4.11 Nutrient agar plates, NVSL medium 10530
- 4.12 *Salmonella* worksheet (**BTYPFMA1102**)

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4.13 Trypticase soy tryptose (TST) broth, NVSL media 10120

4.14 Motility test medium, NVSL media 10403

4.15 Incubator, 38±2 C

5. Procedure

5.1 Dilute grouping antisera with saline containing 0.5% phenol to working dilution as instructed by the manufacturer. Place a drop of Group B, C1, C2, and E antisera on the glass slide or plate. Place approximately the same size drop of antigen (saline suspension of *Salmonella* cells) on the glass slide or plate and mix antigen and antisera with an inoculating wire or edge of loop.

If information on the O group is available on the 10-3 form, testing can begin with antisera against the given O group plus a negative control (see **Appendix 1**).

5.2 Gently rock the slide or plate for 1-2 minutes, watching for an agglutination reaction. The agglutination reaction will be seen as clumping of the cells in the suspension.

5.3 Record the reactions (both positive and negative) on the serotyping worksheet.

5.4 If there is no reaction in the first 4 O groups, groups D and K(18) are tested repeating the procedure in **step 5.1**.

5.5 Test single factor or absorbed antisera according to the reactions as recorded in **step 5.2**. If group B is positive, test single factor 4; if group C1, test 7; if group C2, test 6, 8, and 20; if group D, test 9; if group E, test 10, 15, 19, and 34. Record results as before.

5.6 If there is no reaction in all 6 O groups (**step 5.1**), repeat the procedure in **steps 5.1** and **5.2**, using pools containing the higher O antisera (11-67).

5.7 Record reaction of the pool that agglutinated with the antigen.

5.8 Test components of positive pool with the antigen as before to determine O group.

5.9 If the positive component of the pool is a complex antigen, such as 13,23 or 6,14,24, test the single factor antisera necessary to determine the O group.

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5.10 If the antigen is negative in all of the pools, as well as the first 6 antisera tested, test for agglutination with acriflavin. Record results on worksheet.

5.11 If the antigen is agglutinated by acriflavin, it indicates that the O antigen is “rough.” Smooth to rough variation is a gradual, nonreversible process in which the cell wall is altered and either reacts in many different antisera or none.

5.12 If an antigen is determined to be “rough”, the original isolate is streaked onto a nutrient agar plate. Incubate at 38±2 C overnight. Two colonies are inoculated to BAB slants and TST broth; smooth colonies will be chosen over those appearing rough, unless no smooth colonies are present. Incubate the slants and broth at 38±2 C overnight, and test as before. If there is still no O reaction and acriflavin is positive, the isolate is determined to be untypable due to a “rough O.” H (flagellar) reactions should be determined and reported.

5.13 Continue with H antigen determination (see the current version of **BTYPSOP1103**) on all isolates.

5.14 If there is no O reaction and the H antigens do not react to the polyvalent H antisera, transfer the isolate to a full set of biochemical test media (see the current version of **BTYPSOP1112**) to verify the identification of the isolate as a *Salmonella* species. If the biochemical test results indicate the isolate is not *Salmonella*, it is reported as such. If the biochemical reactions are consistent with those of *Salmonella*, plate the isolate on a nutrient agar plate to try to isolate a pure colony. Pick colonies after overnight incubation at 38±2 C and test as before.

5.15 If the colonies are negative in O and H antisera, test one of the colonies in a full set of biochemical test media as in **step 5.14**. If the biochemical tests indicate the isolate is *Salmonella*, improve the motility by inoculating a tube of motility test medium and determine the H antigens (see the current version of **BTYPSOP1103**).

6. References/Associated Quality Documents

6.1 BTYPFMA1102 *Salmonella* Worksheet

6.2 Edwards and Ewing: Identification of Enterobacteriaceae, 4th Edition, Elsevier. New York, 1986.

6.3 NVSLQSOP0006 Training Employees

7. Revision History

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National Veterinary Services Laboratories	
Document Title: Salmonella Serotyping—Determination of O Antigen	
Author/Position: Brenda Morningstar-Shaw, Microbiologist	Document Number: BTYPESOP1102.05
	Supersedes: BTYPESOP1102.04

7.1 Version 4

Contact information updated. Section 5.1 changed from “Groups 18, B, C1, C2, D and E” to present wording. Section 5.4 added “If there is no reaction in the first 4 O groups, groups D and K(18) are tested repeating the procedure in **step 5.1**” 5.5 was changed from “If group B is positive, test single factor 5” to the present wording. Section 5.11 and 5.12 “Killed antigen” procedure was removed. 5.12 wording added “smooth colonies will be chosen over those appearing rough, unless no smooth colonies are present.” Section 5.14 BTYPESOP1104 Changed to BTYPESOP1112. Appendix 1 change in negative control for “B” from “C2” to “D.”

7.2 Version 3

The document was reformatted and the contact information updated. Disposable inoculating loops are now specified. A Bunsen burner was deleted from the list of supplies.

7.3 Version 2; September 19, 2003

In the **Media and supplies** section, "Saline with 0.5% phenol" (**item 4**) was changed to "0.85% Saline with 0.5% phenol" and **item 16** was added. A second paragraph (beginning "If information...") was added to **step 1** of the **Procedure** section. **Step 5** was changed from "If the antigen is negative in all 6 antisera" to the present wording. The third sentence in **step 10** was changed from "An alcohol-killed antigen should be prepared and tested as this sometimes will agglutinate in the grouping antisera" to the present wording. The sentence "Allow to dry for a few min" was added to **step 10**, and the words "in slide agglutination tests" were added to the last sentence of the paragraph. The word "original" was added to the first sentence of **step 12** and the last sentence was changed from "If H (flagellar) reactions are determined, they can be reported" to the present wording. All references to 37 C in the document were changed to 37-41 C. **Appendix 1** was added.

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Author/Position: Brenda Morningstar-Shaw, Microbiologist	Document Number: BTYPSOP1102.05
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8. Appendices

Appendix 1

Stated O Group	Negative Control
B	D
C ₁	C ₂
C ₂	B
D	B
E	B
18 (K)	C ₁

National Veterinary Services Laboratories	
Document Title: <i>Salmonella</i> Serotyping—Determination of H Antigen	
Author/Position: Brenda Morningstar, Microbiologist	Document Number: BTYPSOP1103.04
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 - 8.2 Appendix 2—Single Factor H Determination
 - 8.3 Appendix 3—Testing for Remaining Phase

Approved: /s/ Linda Cox

Date: 11-08-07

National Veterinary Services Laboratories	
Document Title: <i>Salmonella</i> Serotyping—Determination of H Antigen	
Author/Position: Brenda Morningstar, Microbiologist	Document Number: BTYPSOP1103.04
	Supersedes: BTYPSOP1103.03

1. Purpose/Scope

After *Salmonella* isolates have been logged in and transferred (see **BTYPSOP1101 *Salmonella* Serotyping—Logging In and Transferring**) and the O antigen has been identified (see **BTYPSOP1102 *Salmonella* Serotyping—Determination of O Antigen**), the next step in the serotyping procedure is to determine the H (flagellar) antigen(s). *Salmonella* can be monophasic or diphasic (possessing 2 distinctive H antigens); most serotypes are diphasic. All antigens (O and both H phases) must be identified in order to determine the serotype.

2. Definitions

Not applicable

3. Safety Precautions

Salmonella is a potential human pathogen. Gloves and laboratory clothing must be worn when working with live *Salmonella* cultures. All personnel are trained according to **NVSLQSOP0006** (Training NVSL Employees).

4. Equipment and Materials Required

- 4.1 Automated pipettor, capable of dispensing 20-25 µl
- 4.2 Disposable inoculating loop or wire
- 4.3 Disposable pipette tips
- 4.4 Flagellar antigen-trypticase soy tryptose broth (TST—NVSL medium 10120) culture with 0.85% saline with 0.6% formaldehyde (formalized saline) added (see the current version of **BTYPSOP1101 *Salmonella* Serotyping—Logging In and Transferring**). Allow at least 1 hour after the addition of the formalized saline before using the antigen.
- 4.5 Incubator, 37-42 C
- 4.6 Motility test medium, NVSL medium 10403
- 4.7 Parafilm
- 4.8 Pasteur pipettes, 9 inch, cotton plugged, sterile
- 4.9 Physiological saline, 0.85%, with 0.5% phenol for serum dilution, NVSL medium 30092

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4.10 Physiologic saline, 0.85%, with 0.6% formaldehyde, NVSL medium 30182

4.11 Pipet-Aid XP

4.12 Pipettes, disposable, 10 ml

4.13 Test tube racks

4.14 Tissue culture dish, 60x15 mm (for Gard plates)

4.15 Tubes, 13x100 mm, glass, disposable, plain top

4.16 Tubes, 10x75 mm, glass, disposable, plain top

4.17 *Salmonella* H antisera, rabbit origin, working dilution (usually 1:20)

4.18 Water bath, 45-50 C

4.19 Water bath, 98-102 C

5. Procedure

5.1 Refer to **Appendix 1** to determine which H antisera to test first.

5.2 Mark a 13x100 mm or 10x75 mm tube with culture number and H antisera to be tested and place in a test tube rack. Add 20-25 µl antiserum (using an automated pipettor) and ≈1 ml of antigen to each tube.

5.3 Shake the tubes gently and place the rack in a 45-50 C water bath for 30 minutes to 1 hour.

5.4 Remove the rack from the water bath and look for agglutination in the tubes. Agglutination will be floccular. Tubes should be shaken gently; floccular agglutination may be broken up so it cannot be seen. Record results on the serotyping worksheet. All antigens are "phase 1" except 1 complex (1,2; 1,5; 1,6; or 1,7); e,n,x or e,n,z₁₅; l,w; and z₆ which are usually considered "phase 2".

5.5 Test antigen in single factor (absorbed) antisera if needed. See **Appendix 2**. Repeat **steps 2-4** above, circling the positive single factor on the worksheet.

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5.6 If there is no agglutination after 1 hour, refer to **Appendix 1** for additional H antisera to test.

5.7 Repeat **steps 2-5** above.

5.8 If there is no agglutination after 1 hour, use H pools or polyvalent antiserum to test the antigen. If the polyvalent antiserum is positive, test the antigen in H pools. If one of the pools is positive, test the components of that pool to determine the H antigen. Test single factor antisera if necessary.

5.9 If the antigen is negative in all of the pools or the polyvalent antisera after 1 hour, there are three possibilities that should be considered.

5.9.1 If the isolate is positive in O group D, *Salmonella pullorum* and *S. gallinarum* are nonmotile salmonellae and biochemical testing is required for definitive identification. Refer to the chart in the Fourth Edition of Identification of Enterobacteriaceae for the biochemical tests needed. These are host-adapted serotypes and should be considered a possibility when the source animal is poultry, especially chickens.

5.9.2 The isolate is contaminated or not *Salmonella*. Test the isolate in a full set of biochemical test media (see **BTYPSOP1112** Biochemical Tests for *Salmonella* Serotyping) and determine if the results are consistent with *Salmonella*. Usually there is no reaction in the O group (see **BTYPSOP1102** *Salmonella* Serotyping—Determination of O Antigen).

5.9.3 The isolate is nonmotile or poorly motile. If an O group has been determined, an attempt should be made to improve the motility of the isolate by inoculating a tube of motility test medium and a Gard plate containing no antisera.

5.9.4 If no H antigen can be identified and there is no growth outward in the Gard plate or the tube of motility medium, the isolate is nonmotile. If the isolate is biochemically *Salmonella*, report the O antigen and "nonmotile" for the H antigen. For example: *Salmonella* untypable; 4,5,12:nonmotile.

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5.9.5 Growth outward from the point of inoculation on the Gard indicates motility and can be tested as in **steps 5.11** and **5.12** below. The growth in motility test medium should reach the bottom of the tube before it is removed (from the bottom of the tube) with a 9" Pasteur pipette and added to a tube of TST broth. If there is still no H reaction, another tube of motility test medium can be inoculated by layering agar from the bottom of the first tube on the top of the second tube. A new Gard plate for motility may be inoculated with a loopful of agar from the first motility Gard. Test the antigen after every 2 or 3 passages through motility. After passing through 5 tubes of motility, if there is still no H reaction the isolate shall be considered poorly motile. If the isolate is biochemically *Salmonella*, report the O antigen and "poorly motile" for the H antigen. For example: *Salmonella* untypable 4,5,12:poorly motile. Always use single colonies to pass through motility media and do biochemical testing to confirm identity as *Salmonella*.

5.10 If the H antigen has been determined above, it is necessary to try to reverse the phase in order to identify the remaining H antigen. A Gard plate is used to try to force the phase change to occur. To make the Gard plate, place 1 or 2 drops of antisera against the identified phase and approximately 5 ml of melted motility test media in a 60x15 mm tissue culture dish. Mix the antisera and motility media. Allow to solidify, and use the original isolate to inoculate the Gard plate.

5.11 After overnight incubation at 37-42 C, use a disposable loop to transfer a loopful of agar from the outer edge of growth on the Gard plate to a new tube of TST broth. A Pasteur pipette may also be used to remove agar from the outer edge of growth and add it to a new tube of TST broth. Shake and incubate at 37-42 C for approximately 4 hours or until growth is dense.

5.12 Add formalized saline, allow ≈1 hour before testing, and test in tube agglutination test as before. To determine which antisera to test, check first for the most common serotypes and test for the phase which is missing. See **Appendix 3** for phase 1 options to try if you know phase 2, or phase 2 options when phase 1 was identified first. If results are negative, test for the phase being held back. If that is also negative, try H pools.

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5.13 If the growth from the Gard plate is negative in H pools, the isolate may be contaminated. Make a new Gard with the original isolate and set up a complete set of biochemicals.

5.14 If there is no growth outward from the point of inoculation on the Gard plate, the isolate may be monophasic. Check the antigenic formula in the Kauffmann-White scheme to see if a second phase is needed for identification. If the serotype is monophasic, it can be reported. If a second phase is needed, additional steps must be taken to try to force a phase change.

5.15 Make a Gard plate as before, but seal it with Parafilm before incubation. Leave the Gard in the incubator up to 48 hours and transfer and test as before if there is growth outward from the point of inoculation. Additionally, a tube of motility test medium can be inoculated from the original isolate and incubated at 37-42 C. The tube may be incubated up to 1 week if there are indications of growth through the tube. When (if) the isolate grows to the bottom of the tube, growth is removed from the bottom of the tube using a 9" Pasteur pipette and added to a tube of TST broth. Incubate, add formalized saline, and test as before.

5.16 If still unable to force a phase change, report the antigens that have been identified. For example, "*Salmonella* untypable; 4,5,12:i:-" would indicate an isolate that was missing phase 2.

5.17 If the results of the phase change indicate the presence of two phase 1 or two phase 2 antigens and there is no serotype listing in the Kauffman-White scheme, the isolate may be a mixed culture containing more than one serotype. Repeat **steps 10-12**.

5.18 If the results still indicate a mixed culture, two options are available.

5.18.1 Streak the isolate on a nutrient agar plate, incubate at 37-42 C overnight, and pick two colonies to BAB slants and TST broth. Test as before, beginning with identification of O antigens, and proceeding with the identification of the H antigens for each colony.

5.18.2 Report the isolate as "Multiple Serotypes."

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5.19 If the antigenic formula is one that has never been reported by your laboratory or is not listed in the Kauffman-White scheme, assume that the isolate may be mixed and proceed as in **5.18.1**. If the results are the same as before, inoculate a full set of biochemicals and report the serotype. If the serotype has not been reported in the Kauffman-White scheme, submit the colony to CDC in Atlanta, Georgia, for confirmation of a new serotype.

6. References/Associated Quality Documents

6.1 Edwards and Ewing: Identification of Enterobacteriaceae, 4th Edition, Elsevier. New York, 1986.

6.2 **BTYPSOP1101** *Salmonella* Serotyping—Logging In and Transferring

6.3 **BTYPSOP1102** *Salmonella* Serotyping—Determination of O Antigen

6.4 **BTYPSOP1112** Biochemical Tests for *Salmonella* Serotyping

6.5 **NVSLQ SOP0006** Training NVSL Employees

7. Revision History

Version 4

The document was reformatted. Minor changes were made to **sections 4** and **5**. References were added.

Version 3

The document was reformatted and the contact information was updated. Disposable inoculating loops are now specified. The third sentence in **step 15** of the procedure was changed to eliminate the use of 3 drops of antisera in the motility medium and to specify an incubation temperature. In **Appendix 1**, O group B, the H antisera "l,v; e,h" were moved from the 1st group to the 2nd group of H antisera and the phrase "or H pools" was added to the 2nd group of H antisera for O group D.

Version 2; September 19, 2003

Water bath temperatures were changed from 50 C to 45-50 C throughout the document. The references to 0.2 ml glass pipettes were changed to a Rainin EDP3 pipettor or Pipetman P200

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throughout the document. The volume of antiserum used was changed from 0.02 ml to 20-25 µl throughout the document. A 37-42 C incubator was added to the list of supplies, and incubation temperatures were changed from 37 C to 37-42 C throughout the document. In **section 9.1**, "If the isolate is positive in O group D," was added to the beginning of the first sentence. In **section 9.2**, the sentence "Usually there is no reaction in the O group (see the current version of **BTYPSOP1102**)" was added to the end of the paragraph. In **section 9.3**, the phrase "If an O group has been determined," was added to the beginning of the second sentence. The sentence "A new Gard plate for motility may be inoculated with a loopful of agar from the first motility Gard" was added. "Always use single colonies to pass through motility media and do biochemical testing to confirm identity as *Salmonella*" was added to the end of the section. The following changes were made in the procedure: In **step 10**, "a drop" was changed to "1 or 2 drops." In **step 11** the sentence "A Pasteur pipette may also be used to remove agar from the outer edge of growth and add it to a new tube of TST broth" was added. In **step 12** the phrase "in antisera" in the first sentence was changed to "in tube agglutination test." The following was added to the end of the section: "Or phase 2 options when phase 1 was identified first. If results are negative, test for the phase being held back. If that is also negative, try H pools." **Step 13** was added and subsequent steps renumbered accordingly. In **step 17**, "or phase 2 antigens" was changed to "or two phase 2 antigens." In **step 18**, "are the same as described in step 16" was changed to "still indicate a mixed culture."

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8. Appendices

Appendix 1—*Salmonella* H Antigens

When testing for the flagellar or H antigens, use 13x100 mm tubes with 20-25 µl antisera (diluted 1:20 with saline with 0.5% phenol) and 1 ml antigen. The tubes are incubated in a 45-50 C water bath for 30 minutes to 1 hour.

O Group	H Antisera
B	1 st i; 1,2; r; f,g 2 nd d; z; b; m,t; e,n,x; l,v; e,h
C ₁	1 st g,m,s; m,t; z ₂₉ ; 1,5; r; z ₁₀ 2 nd b; d; l,w; z ₃₈ ; e,h; e,n,x; k; y; c Isolate from swine: c and 1,5 first; <i>choleraesuis</i> biochemicals (BTYPSOP1104 Phage Typing of <i>Salmonella typhimurium</i>) if either is positive.
C ₂	Single Factor 8 positive, 6 + or - 1 st e,h; d; k; 1,2; z ₁₀ ; e,n,x 2 nd l,v; r; z ₄ ,z ₂₄ ; i; z ₆ Single Factors 8 and 20 positive z ₄ ,z ₂₄ ; i; z ₆ ; z ₁₀
D	1 st g,m; l,v; 1,5; f,g 2 nd Polyvalent (PV) or H pools—If negative, inoculate <i>pullorum</i> biochemicals for poultry isolates and full biochemicals for other species (BTYPSOP1104 Phage Typing of <i>Salmonella typhimurium</i>)
E ₁ (10)	1 st e,h; l,v; 1,6 2 nd y; z ₁₀ ; g,m,s
E ₂ & E ₃ (15, 34)	e,h; l,v; 1,6; y; z ₁₀ ; g,m,s
E ₄ (19)	1 st g,s,t; z ₂₇ ; z ₄₃ 2 nd y; l,w; i; z ₆
K (18)	z ₄ ,z ₂₃ Biochemical test for Arizona (BTYPSOP1104 Phage Typing of <i>Salmonella typhimurium</i>)
13,22 (G ₁)	z; 1,6

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13,23 (G₂) f, g; z₂₉; d; z; l, w

35 f, g; z₄, z₂₃

40 b; e, n, x

If there is no agglutination in the antisera tested, try PV. If PV positive, try H pools; if PV negative, do complete biochemical testing.

H Pools—Use 10x75 mm tubes, 20–25 µl antisera, and 0.5 ml of antigen.

<u>Pool</u>	<u>Components</u>
A	a; b; c; d
B	e, h; e, n, x; i; k
C	"g" complex
D	l, w; r; y; z
E	z ₄ , z ₂₃ ; z ₆ ; z ₁₀ ; z ₂₇ ; z ₂₉
F	z ₃₅ ; z ₃₈ ; z ₄₃ ; z ₅₂ ; z ₅₃
H	"1" complex
G1	z ₃₆ ; z ₃₇ ; z ₃₉ ; z ₄₁ ; z ₄₂ ; z ₄₄
G2	z ₄₅ ; z ₄₆ ; z ₄₇ ; z ₄₈ ; z ₄₉ ; z ₅₀ ; z ₅₉

Use Pools G1 and G2 only when other pools are negative and PV is positive.

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Appendix 2—Single Factor H Determination

When testing for single factor H antigens, use 10x75 mm tubes with 20-25 µl diluted antisera and 0.5 ml antigen and incubate as before.

H antigen	H single factors
e,h	h
e,n,x or e,n,z ₁₅	n, x, z ₁₅
"g" complex	f, m, s, t except for "O" Group D check f, m, s, t, p, q, u For higher O's, check f, m, s, t, z ₅₁
l,v or l,w	1 st v, w 2 nd z ₁₃ , z ₂₈ Group E ₁ & E ₂ : v, w, z ₁₃ Group D: v, z ₂₈
z ₄ , z ₂₃ OR z ₄ , z ₂₄	z ₂₃ , z ₂₄ , z ₃₂
l complex	2, 5, 6, 7 depending on O group B: 2, 5, 7; C1: 5, 6; C2: 2, 5; D: 5, 6; E: 5, 6, 7, (2)

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Appendix 3—Testing for Remaining Phase

Check in book for most common serotypes or check list below when going from phase 2 to phase 1.

B	Try	C ₁	Try	C ₂	Try	D	Try
1,2	e,h; i; r; (b)	1,5	k,r,y (c; e,h)	1,2	d; e,h; l,v	1,5	l,v (a; e,h)
1,5	e,h; z	1,6	e,h	1,5	d; e,h; k; r	e,n,x	a
1,7	d; l,v; z	1,w	b,d	e,n,x	z ₁₀		
e,n,x	e,h	e,n,x	y (a)	1,7	l,v		
e,n,z ₁₅	e,h; l,v	e,n,z ₁₅	e,h; z ₁₀	z ₆	i		
E ₁	Try	E ₂	Try	E ₃	Try	E ₄	Try
1,5	e,h; l,z ₁₃ ; y; z ₁₀	1,5	e,h; y; z ₁₀	1,5	e,h; y; z ₁₀	1,w	y,b
1,6	e,h; l,v	1,6	e,h; l,v	1,6	e,h	z ₆	i
1,7	l,v	1,7	l,v	1,7	l,v	z ₄₃ , z ₂₇	g,s,t
1,2	y						
1,w	e,h						