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| Shigella Antisera Typing |
| **Purpose** | This procedure provides instruction for the performance of SHIGELLA TYPING***Shigella sp*.** isolates are further characterized and grouped according to their somatic, or O, antigen. Antigen typing is necessary to provide serological confirmation after biochemical identification of *Shigella sp*. isolates taken from microbial culture specimens. Isolates can be further classified into groups A(*S. dysenteriae*), B(*S. flexneri*), C(*S. boydii*), and D(*S. sonnei*). |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture plate reading. Serological testing is performed when presumptive biochemical testing is suggestive of *Shigella* or to confirm a complete biochemical identification of the same. |
| **Work-up Codes** | SHGA; SHGB; SHGC; SHGD |
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|  | **Reagents** | **Supplies** | **Equipment** |
| **Materials** | * Shigella Group A grouping antisera
	+ Cardinal Health product number 4341025
* Shigella Group B grouping antisera,
	+ Cardinal Health product number 4341027
* Shigella Group C grouping antisera
	+ Cardinal Health product number 4341028
* Shigella Group D grouping antisera
	+ Cardinal Health product number 4341031
	+ Antisera are stable until product expiration date on label when stored at 2-8°C.
	+ Do not expose reagents to room temperature for long periods of time.
	+ Discard any antiserum which becomes cloudy during storage.
	+ Do not subject antisera to repeated freezing and thawing. Such treatment is detrimental to the antibody content.
 | * Sterile 0.85% Saline
* Wooden applicator sticks
* Disposable petri dish
* China Marker
* Falcon tubes
 | * Orbital mixing device
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| Sample | * Well-isolated 18-24 hour old colonies of suspected or biochemically confirmed *Shigella sp*. isolates.
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. Biohazard Containment
2. Safety in the Microbiology/Virology Laboratory
* Biohazardous Spills
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| **Quality Control** | 1. Perform quality control with each new lot or shipment prior to use. Perform quality control semi- annually following DESK3 Maintenance Schedule. Refer to MC 8.6f Shigella Typing Quality Control.
2. If there is a QC failure, document observation, notify Microbiology Supervisor and call BD technical service at 1-800-638-8663. Do not report patient results until the problem has been resolved.
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| **Procedure** | Make a heavy (milky) suspension of suspected or known *Shigella* *sp*. isolates in 0.85% saline solution. Alternatively, a drop of saline and a small portion of a well-isolated colony may be used.Place one drop of appropriate antiserum in each well of a ringed agglutination slide.Add one drop of bacterial suspension to each well containing *Shigella sp.*antiserum.1. Mix thoroughly with an applicator stick to achieve a homogenous mixture.
2. Gently rock the agglutination slide for one minute or use the mechanical rotator.
3. Examine for agglutination, over the MicroScan light box.
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| **Interpretation/ Results/Critical Values** | 1. Read the results as follows:

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| 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 |

1. Positive result: 3+ or greater agglutination is a positive result.
2. Negative result: 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.
3. A partial (less than 3+) or delayed agglutination reaction should be considered negative.
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| **Limitations** | * Correct interpretation of serological reactions depends on culture purity, morphological characteristics, and biochemical reactions that are consistent with ***Shigella sp.***
* Serological methods alone are not sufficient to identify ***Shigella sp.*** isolates.
* Capsular (K) antigens can mask the somatic antigen found in ***Shigella***.
	+ Suspicious isolates of ***Shigella*** giving weak or negative results on initial typing should be heated in a boiling water bath for 15-30 minutes and retested.
* Excessive heat from external surfaces may prevent a smooth suspension, or may cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
* Rough culture isolates do occur, and will agglutinate spontaneously, causing agglutination of the negative control reaction (auto-agglutination). Smooth colonies must be selected and tested in serological procedures.
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| **Method Performance Specifications** | * All materials and equipment must be at room temperature at the time of test performance.
* Adhere strictly to the time limitations on the tests.
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| **Result Reporting** | 1. Record results in Sunquest MRE in the Culture Entry tab. Click on the Workups button. Enter results by using the customized keyboards or by entering a code in the result box.

Observations: 1. PRESUMPTIVE SHIGELLA SONNEI (SEROGROUP D) ISOLATED. Biochemical confirmation to follow.Workups: Workup #1 Workup Components Med : HE SHGD : POS Desc : NLF SC : SB SMDH : DONEIf Shigella typing is positive, report as: “Presumptive Shigella species, Biochemical confirmation to follow.” * Report appropriate serotype in Sunquest MRE Culture Entry tab, Observations, using the following SUNQUEST MO codes: SHDY (A), SHFL (B), SHBO (C), SHSO (D).

Set up Vitek GNI and GNS 69 for complete ID and AST. Send isolate to MDH and call result to caregiver. If typing is negative, set identification on Vitek |
| **References** | 1. Difco Laboratories (1998). *The Difco manual, 11th ed*. Sparks, MD: Becton, Dickinson and Company
2. Becton, Dickinson and Company. (9/2014). *Shigella antisera for the serological identification of Shigella: Product insert*. Sparks, MD: Becton, Dickinson and Company
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.1 | K. Renner | 6/2006 | Initial Version |
| 1.2 | R. Adra | 7/28/2009 | Revised to Micro Procedure Template Format  |
| 1.3 | Jessica Craig | 08/11/2010 | Online format |
|  | 1.4 | Becky Carlson | 10/26/2010 BJC | Combined Salmonella and Shigella antisera typing procedures. Discontinued salmonella subtyping  |  |  |
| 2 | Becky Carlson | 4/25/2015 | Re-numbered from MC 928 for CMS load |
| 3 | Susan DeMeyere | 9/15/2017 | Removed Salmonella typing and updated QC information.  |
| **Archived by:** |  | **Archived Date:** |  |