|  |
| --- |
| *B. pertussis* and *B. parapertussis* FA (Culture Confirmation) |
| **Purpose** | This procedure provides instruction for performing *Bordetella pertussis* & *Bordetella parapertussis* FA stain culture confirmation. Pertussis (whooping cough) is a highly contagious, acute infection of the upper respiratory tract caused primarily by *B. pertussis* and less commonly by *B. parapertussis. B. parapertussis* has a less severe clinical presentation in both duration and symptomsand accounts for less than 5% of all pertussis cases. *Bordetella holmesii* has also been reported to cause a pertussis-like illness. Infants with pertussis may present with choking and apnea, while cough may be absent. Infants are more likely than other age groups to suffer from severe disease and complications, including cyanosis and pneumonia, and to die. Treatment in the early stages of disease reduces the severity of illness and spread of the disease. Early laboratory diagnosis is essential in the control and prevention of the disease. |
| **Policy Statements** | This procedure applies to microbiologists who perform culture set-up and/or plate reading.  |
|  |  |  |  |
| **Materials** | **Reagents** | **Supplies** | **Equipment** |
|  | * FA *B.pertussis* conjugate
* BD catalog # 223591
* FA *B. parapertussis* conjugate
* BD catalog # 223781
* FA Buffer
* BD catalog # 223143
* FA mounting medium
	+ Diagnostic Hybrids catalog # 01-00212015
* 95% alcohol
 | * FA Slides
* Glass coverslip 22x22mm
* Staining tray
* DI water
* Disposable inoculating loop
 | * Fluorescence microscope filtered for FITC (490nm/520nm band pass)
 |
|  |
|  | **Reagent Storage Requirements** |
|  | * On receipt, store lyophilized FA conjugate at 2 to 8ºC until expiration date. After rehydration, store aliquots of undiluted conjugate at -20ºC or below.
* Store dehydrated FA buffer at room temperature. After rehydration, store at 2 to 8ºC
* Allow reagents to come to room temperature before use.
* Lyophilized product deterioration: Expiration date applies to the reagents in their intact container as directed. Do not use if either conjugate or buffer reagent appears caked or discolored.
* Reconstituted product expiration: Discard rehydrated buffer 1 year after rehydration or if cloudy. Frozen-rehydrated FA conjugate can be used until original expiration date.
* Reconstituted product deterioration: Solutions showing turbidity or mold growth should be discarded.
 |
| **Sample****Special Safety Precautions** | * Culture isolates: colony picked from a Regan-Lowe plate (RL).

Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. [*Biohazard Containment*](https://starnet.childrenshc.org/References/labsop/mcvi/safety/mcvi-3.1-biohazard-containment.pdf)
2. [*Safety in the Microbiology/Virology Laboratory*](https://starnet.childrenshc.org/References/labsop/mcvi/safety/mcvi-3.2-safety-in-the-microbiology-lab.pdf)
* [*Biohazardous Spills*](https://starnet.childrenshc.org/References/labsop/mcvi/safety/mcvi-3.4-biohazardous-spills.pdf)
 |
| **Quality Control** | 1. *B. pertussis* FA conjugate
	1. Positive Control: *Bordetella pertussis* ATCC 8467, 4+ reaction
	2. Negative Control: *Bordetella parapertussis* ATCC 9305, <1+ reaction
2. *B. parapertussis* FA Conjugate
	1. Positive Control: *Bordetella parapertussis* ATCC 9305, 4+ reaction
	2. Negative Control: *Bordetella pertussis* ATCC 8467, <1+ reaction
3. Perform QC with each new lot or shipment before put into service. Record results in QC manual.
4. **Perform QC with each patient run.** Record results on Desk 2 QC checklist.
5. If there is a QC failure, document observation, notify supervisor, and call BD technical service at 1-800-638-8663.
 |
| **Reagent Preparation** | 1. FA Conjugate preparation
2. Rehydrate with 5.0 mL of sterile distilled water.
3. Rotate vial gently to dissolve the contents.
4. Aliquot undiluted conjugate (50-100µL) in 12x75 tubes for storage.
5. Titer conjugate in FA buffer to determine the working dilution, using a culture of *B. pertussis* ATCC 8467. Make dilutions as follows:

|  |  |  |
| --- | --- | --- |
| *Dilution of conjugate* |  | *Fluorescence*  |
| 1:5 | Add 0.1 ml conjugate to .4 ml buffer | 4+ |
| 1:10 | Add 0.1 ml conjugate to .9 ml buffer | 4+ |
| 1:20 | Add 0.1 ml conjugate to 1.9 ml buffer | 4+ |
| 1:40 | Add 0.1 ml conjugate to 3.9 ml buffer | 2+ |

1. If the last 4+ dilution is 1:20, select one less dilution. The working dilution would then be 1:10.
2. FA Buffer Preparation
	1. Rehydrate a 10 gram vial with 1 liter of type 1 water.
	2. pH the buffer (The pH should be 7.2 ± 0.5).
 |
| **Procedure** | 1. Culture Isolates
	* 1. Pick isolated colony from a RL plate. Colonies will appear round, domed, mercury-silver colored and shiny.
		2. Emulsify colony in a drop of sterile DI water. Do not make spot too dense. Adjust to approximately a 1 McFarland standard.
		3. Spot the colony suspension to an FA slide. Air dry.
2. Quality Control Slides
3. Prepare the positive and negative control slides from the QC cultures of BPER and BPAR following the procedure for the patient isolates.
4. Staining Procedure
5. Fix slides with 95% alcohol for 1 minute.
6. Add FA buffer to a frozen aliquot of BPER FA conjugate to make the determined working dilution.
7. Add FA buffer to a frozen aliquot of BPAR FA conjugate to make the determined working dilution.
8. Add 1 drop of each prepared BPER and BPAR FA conjugate to the rings on the slide for each patient and the positive and negative QC slides.
9. Incubate in a “wet box” for 30 minutes at room temperature.
10. Gently wash the excess conjugate off into the mercury waste jar using FA buffer.
11. Gently wash the FA buffer into the mercury waste jar using type 1 water.
12. Use caution to prevent possible cross contamination of slides.
13. Air dry slides in the dark.
14. Add 1 drop of FA mounting media on each ring and coverslip.
15. Examine each smear using a fluorescent microscope under 50X or 100X oil immersion.
16. Record presence or absence of small fluorescent coccobacilli.
 |
| **Interpretation/ Results/Critical Values** | 1. *B. pertussis* and *B. parapertussis* will appear as brightly fluorescent coccobacilli with a clear cut periphery and a dimmer staining center. Record the intensity of fluorescence as follows:

|  |  |
| --- | --- |
| 4+ | Maximum fluorescence; brilliant apple-green peripheral staining. |
| 3+ | Bright apple-green peripheral staining. |
| 2+ | Definite, but dull, apple-green peripheral staining. |
| 1+ | Barely visible peripheral staining. |
| Negative | Complete absence of fluorescence. |

1. Positive Control: 4+ reaction
2. Negative Control: <1+ reaction
3. Patient smears: 3+ or 4+ is considered a positive result.
4. POSITIVE FA’s must be reviewed by a 2nd technologist before reporting.
5. If the positive control is less than 3+ and the negative control is >1+, the conjugate may have deteriorated or the pH of the buffer or mounting media may not be correct. Repeat testing using new reagents.
6. If background fluorescence compromises the quality of the smear, repeat test using Evans blue counterstain.
 |
| **Method Performance Specifications** | 1. Cultures that are negative for *B. pertussis* should be tested for *B. parapertussis* if the colony morphology is consistent.
2. Questionable FA morphology should be confirmed with gram stain.
 |
| **Result Reporting** | 1. Record results in Sunquest MRE in the Culture Entry tab and Workup section. An example is as follows:

Observations: 1. 3+ BORDETELLA PERTUSSIS2. \*\*Called to and read back by Dr. Who 7/8/05 @ 1400Workups: Workup #1 Workup Components Med : RL SC : RL for MDH Desc : SML GMS : GNR, SML Id : BPER FA : BFAP, FANB1. Call POSITIVE culture results to the ordering provider.
 |
| **References** | 1. “BD Difco™ FA Bordetella pertussis and FA Bordetella parapertussis”, BBL circular 51317, Oct. 2014, Becton Dickenson and Co., 7 Loveton Circle, Sparks, MD 21152.
2. Leber, Amy. *Clinical Microbiology Procedures Handbook*, 4th edition. Vol. 1-3 (Section 3.11.6). 2016. American Society for Microbiology, Washington D.C., 20036.
 |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Competency** |
| 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
 |
|  |  |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | 1 | Pat Ackerman | 1992 | Initial Version |
| 1.1 | Pat Ackerman | 02/03/1992 | Updated Sunquest 6.2 result reporting and recording. Revised SRPT and CORR reporting statement. Added hyperlinks. |
| 1.2 | Pat Ackerman | 08/03/2005 | Added the review of positives by a 2nd tech before reporting BFAP |
|  | 1.3 | Becky Carlson | 07/2012 | *B. pertussis/parapertussis* Direct FA test (from Nasal swab or Nasal wash) no longer performed. |  |  |
| 1.4 | Becky Carlson | 07/24/2013 | Added reagent deterioration and expiration instructions |
| 1.5 | Tina Gronquist | 07/28/2014 | Updated into online format. |
| 2 | Becky Carlson | 4/18/2015 | Re-numbered from MC 705 |
| 3 | Susan DeMeyere | 8/28/2019 | Changed QC to perform with each patient run.  |
| **Archived by:** |  | **Archived Date:** |  |