|  |
| --- |
| GC Culture |
| **Purpose** | This procedure provides instructions for GC CULTURE for the Microbiology laboratory. |
| **Policy Statements** | This procedure applies to Microbiologists/Virologists who perform culture set-up and plate reading. |
| **Principle and Clinical Significance** | *Neisseria gonorrhoeae* is the causative agent of gonorrhea. Although it is primarily a pathogen of the urogenital tract, *N. gonorrhoeae* is considered clinically significant regardless of the site of isolation. *N. gonorrhoeae* is generally transmitted sexually. Cases of gonococcal infection in the newborn are the result of ocular contamination during delivery. The collection of appropriate specimens for diagnosis of gonococcal infection is dependent on the gender and sexual practices of the patient and on the clinical presentation. Infections usually are localized to the mucosal surfaces in the initial exposure to the organism, e.g., cervix, conjunctiva, pharyngeal, anorectal area or urethra of males. Localized infections can be acute with a purulent discharge or they may be asymptomatic. Disseminated disease may appear in 0.5 to 3% of infected individuals.A good selective medium is required for reliable isolation of gonococci from specimens that may contain a variety of microorganisms, including saprophytic *Neisseria* sp. Methods for the identification of *N. gonorrhoeae* from extra-genital sites and cultures from children must be chosen and performed with the utmost care, since a positive report can have far-reaching psychological and medico-legal implications. |
| **Test Code** | GC |
| **Materials** | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram Stain reagents
* Oxidase
* 3% hydrogen peroxide
* Vitek® NH cards
 | * Glass slides (plain)
 | * CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
 | * Chocolate agar (CHOC)
* Modified Thayer-Martin agar (MTM)
 |
| Sample | 1. Acceptable specimens
* Swab of vagina, cervix, urethra, rectum, throat, endocervix
* joint fluid
* newborn eye
* first few drops of voided urine (men only)
1. SDES codes/Specimen type
* CERV – cervix
* EYE-LT – left eye
* EYE-RT – right eye
* GCT – throat, GC only
* JF – joint fluid
* RECT – rectum
* UR – urine
* URE –urethra
* VAG – vagina
1. Specimen Collection and Transport
* Refer to [\\kidsnet.childrenshc.org\chcdfs\dept\Lab Procedures\Microbiology\1NEW Micro Procedure Manual. (same as in Starnet)\Specimen Collection Procedures\GC Culture.doc](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CSpecimen%20Collection%20Procedures%5CGC%20Culture.doc)Lab Test Directory – GC Culture (*Neisseria gonorrhoeae* only)
1. Specimen assessment
* Refer to the Specimen rejection section of Lab Test Directory – GC Culture (*Neisseria gonorrhoeae* only)
 | [Lab Test Directory – GC Culture (*Neisseria gonorrhoeae* only)](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/032996.asp) |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies***:***1. [*Biohazard Containment*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20201%20%20%20Biohazard%20Containment.doc)
2. [*Safety in the Microbiology/Virology Laboratory*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20202%20%20%20Safety%20in%20the%20Microbiology%20Lab%20Policy.doc)
* [*Biohazardous Spills*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20204%20%20%20Biohazardous%20spills.doc)
 |
| **Procedure** |  |  |
| **Inoculation** |
| 1. | Warm all media before inoculation. |
| 2. | Label all plates properly with the patients name, accession number and date. |
| 3. | Inoculate the media in the order of the least selective first to prevent carryover of inhibitory substances to another medium. Refer to the Sunquest specimen label for the order of inoculation. |
| **Specimen processing** |
| **Swab Specimens** |
| 1. | Roll swab across the upper quadrant of the CHOC and MTM, touching all surfaces of the swab. |
| 2. | Streak plates semi quantitatively for primary isolation. |
| 3. | Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool. |
| 4. | Pass the loop through the edge of the first quadrant approximately 4 times while streaking into the second quadrant. Continue streaking in the second quadrant without going back into the first quadrant 3-4 times. |
| 5. | Flame loop again, turn the plate another quarter of a turn, and pass the loop through the edge of the second quadrant approximately four times while streaking into the third quadrant. Continue streaking in the third quadrant without going back into the second quadrant 3-4 times. |
|  |  |
| **Urine—MALE only---** first morning-voided specimen. |
| 1. | Spin freshly collected urine (first few drops) at 2500 rpm for 10 minutes. |
| 2. | Decant supernatant. |
| 3. | Using a sterile dispo-pipette, inoculate CHOC and MTM with the sediment. |
| **Incubation** |  |
| 1. | Incubate CHOC and MTM in 3 – 5% CO2 at 35ºC. |
| **Culture examination** |
| **Day 1.** |  |
| 1. | Examine CHOC and MTM plates for *N. gonorrhoeae.* |
| 2. | Gram stain suspicious colony types and perform initial identification procedures, i.e., Gram stain, catalase, oxidase, etc. *N. gonorrhoeae* is a gram-negative diplococci that is oxidase and catalase positive. |
| 3. | The presence of yeast may be significant. Report if present. **Do not ID**. |
| 4. | Set up definitive biochemical or identification procedures on suspicious organisms if well isolated. |
| 5. | Subculture organisms that are not well isolated to appropriate media for further work-up. |
| 6. | Reincubate primary plates and subcultures for an additional day. |
| 7. | Report preliminary results. See Reporting section. |
| **Day 2.** |
| 1. | Examine primary plates from the previous day for additional suspicious microorganisms. |
| 2. | Set up additional tests as needed. |
| 3. | Send updated report. See Reporting section. |
| **Day 3.** |
| 1. | Examine primary plates at 72 hours. |
| 2. | Complete identification procedures until all suspicious isolates are finished. |
| 3. | Send updated report and finalize.  |
| **Procedure Notes** |
| 1. | Do not perform AST on *N. gonorrhoeae* unless there is a case of therapeutic failure. Refer to Mayo Medical Labs for AST, MML test code ZMMLS for AST. |
| 2. | Do not perform beta-lactamase test because current therapies are not affected by beta-lactamase production. |
| 3. | If *N. gonorrhoeae* is isolated, save a representative primary plate in CO2 at 35ºC for 7 days in case a physician calls for further studies. Freeze *N. gonorrhoeae* if isolated from a prepubertal child for future reference. |
| **Result Reporting** | Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box. Report results semiquantitatively, i.e., 1+, 2+, 3+ or 4+.

|  |  |  |  |
| --- | --- | --- | --- |
| Quantity | 1st quadrant# colonies | 2nd quadrant# colonies | 3rd quadrant# colonies |
| 1+ | <10 |  |  |
| 2+ | >10 | <5 |  |
| 3+ | >10 | >5 | <5 |
| 4+ | >10 | >5 | >5 |

 |
| **Negative Results** |
| 1. | Negative cultures for *N. gonorrhoeae*: Use MO codes **NGC1, NGC2** and **NGC3.** Report yeast if present**.** Yeast ID not required.Observations: 1. NO NEISSERIA GONORRHOEAE ISOLATED AFTER 1 DAY 2. 1+ YEAST No further identificationWorkups: Wkup # 1 Workup Components Med : CHOC GRAM : YST Desc : WH  ID : YEAS  |
| **Preliminary Positive Results—pending biochemical confirmation** |
| 1. ---**Sexually active teenager/young adult--** |
| a. | Presumptive *Neisseria gonorrhoeae* isolated, biochemical testing to follow. **PGC-BCTF.** This code may be used if the isolate is from a genital site of a sexually active teenager/young adult, and is oxidase positive, a gram-negative diplococci, and growing on MTM with confirmation pending.  |
| b. | Notify RN, CNP or physician of presumptive positive culture results. Document in computer, first name, first initial of last name, credentials, date and time. |
| c. | Perform MALDI or Vitek® NH card to identify. |
| d. | If *N. gonorrhoeae* is isolated from a sexually active teen-ager / young adult, one identification test is acceptable. |
| **2. ---NOT sexually active--- Prepubertal child (≤ 12 y.o.).**  **MCRC, Cornerhouse or sexual assault patient.** |
| a. | **Do not** use the code **PGC** for preliminary results before MALDI or Vitek® NH card is done, if the culture is from MCRC, Cornerhouse , sexual assault or prepubertal child (≤ 12 y.o.). Report as “Gramnegative cocci, further identification to follow”, MO codes: **GNC-FID**. |
| b. | Notify RN, CNP or physician of presumptive positive culture results (GNC-FID) by phone. Document in culture work-up, first name, and first initial of last name, credentials, date and time.  |
| c. | If *N. gonorrhoeae* is isolated from a prepubertal child (≤ 12 y.o.) or is from a sexual assault, **two different tests must be used for identification.** ---Vitek® NH card or MALDI---Send isolate to MDH for confirmation if Vitek® NH card or MALDI = GC. |
| d. | If MALDI or Vitek® NH card = GC: report “Presumptive *Neisseria gonorrhoeae* isolated,. Sent to MDH for confirmation: MO codes – **PGC-MDHC.** |
| e. | Notify RN, CNP or physician of presumptive GC results (**PGC-MDHC**) by phone. Document in Sunquest Culture Results, first name, first initial of last name, credentials, date and time.  |
| **3. Other sites** |
| a. | Specimens from other sites such as throat have a higher probability of isolation of *N. meningitides*, *N. cinerea,* and *Moraxella catarrhalis* and should be reported as “Culture in progress” , MO codes: **CIP**. |
|  | b. | Perform MALDI or Vitek® NH card to identify. |
| c. | If *N. gonorrhoeae* is isolated from a sexually active teen-ager / young adult, one identification test is acceptable |
| d. | If *N. gonorrhoeae* is isolated from a prepubertal child (≤ 12 y.o.) or is from a sexual assault, **two different tests must be used for identification.** ---MALDI or Vitek® NH card ---Send isolate to MDH for confirmation if MALDI or Vitek® NH card = GC. |
| e. | See reporting guidelines above 1a and b., 2a, b and d, e., depending on the patient age, and/or clinic for reporting preliminary results. |
| **Positive Cultures** |
| 1. | Positive cultures for *N. gonorrhoeae*: Observations: 1. 3+ NEISSERIA GONORRHOEAE MO code – **NGON** 2. \*\*Called to Dr. Spring 12/4/04 @1500. Workups: Wkup # 1 Workup Components Med : CHOC OXI : POS Desc: TAN GMS : NEIS Id: NEIS VID : 1  |
| 2. | Notify patient’s caregiver if *Neisseria gonorrhoeae* is isolated. Document in Sunquest Culture Results, first name, first initial of last name, credentials, date and time.  |
| 3. | If the culture is overgrown with other organisms such as *P. mirabilis* or yeast, report as follows: **UDET-NGON**. Heavy growth of yeast is inhibitory to *N. gonorrhoeae.*Observations: 3. DUE TO BACTERIAL OVERGROWTH, UNABLE TO DETERMINE THE PRESENCE OF NEISSERIA GONORRHOEAE |
| 4. | Review Culture Summary for accuracy before filing report.  |
|  | 5. | If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report. The code SRPT (supplementary report) must be used in SREQ or *Culture Observations* as follows:Updated or new culture information: In the *Culture Entry* tab, enter SRPT on an observation line followed by new results.Requests for additional testing: In the *Misc. Updates* tab, enter SRPT in SREQ followed by the request.Re-final the culture when identifications and/or testing are complete. |
| 6. | If a culture requires a correction, the code **CORR** (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to the procedure [Labeling Errors/Specimen Mix-ups and Correcting Patient Data.](file://\\kidsnet.childrenshc.org\chcdfs\dept\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MC%20100%20%20%20%20Quality,Spec.%20mgmt.,Labeling,Proc.,Sendout%20Results,Billing,%20PT%20testing,Addl%20Projects\MC%20102%20%20%20Labeling%20Errors,%20Specimen%20mixups,%20Corrected%20reports.doc) |
| **Limitations** | 1. Some strains of *N. gonorrhoeae* are inhibited by the vancomycin in the MTM. It is important to view the CHOC carefully for oxidase positive organisms.
2. False-negative oxidase reactions from CHOC have occurred on *Neisseria* sp. .
3. A single negative result produced by any of the confirmatory tests does not rule out the identification of *N. gonorrhoeae.* Further confirmatory testing should be performed. More than one confirmatory method is essential for potential cases of child abuse, since nearly every method has errors.
 |
| **Method Performance Specifications** | 1. Swabs are acceptable for *N. gonorrhoeae* if the specimen will be plated within 6 hours.
2. Cultures must be performed in all medico-legal cases involving prepubescent children. Nucleic acid testing can be performed in addition to culture. In the United States, culture identification of *N. gonorrhoeae* is the only definitive method of diagnosis from a legal standpoint.
3. *N. gonorrhoeae* is sensitive to cold temperatures and drying.
4. Heavy growth of yeast is inhibitory to *N. gonorrhoeae.*
5. Because *N. gonorrhoeae* is very labile, a negative culture does not rule out infection.
 |
| **References** | 1. Forbes, B.A., et al., Bailey & Scott’s *Diagnostic Microbiology*, twelfth edition, 2007. Mosby, Inc., St. Louis, MO., pg. 452-453.
2. Versalovic, James, et al, *Manual of Clinical Microbiology*, 2011, ASM press, American Society for Microbiology, Washington, D.C.
3. Pezzlo, M., Section 2, Aerobic bacteriology, 2.7, Processing and interpretation of genital cultures, *In* H.D. Isenberg (ed) *Clinical Microbiology Procedures Handbook*, 1994, American Society for Microbiology, Washington, D.C., pg. 81-89.
4. BBL Quality Control and Product Information Manual for Plated Media, L007392, Rev. 06. January 2001, pg. 81-82.
5. Kellogg, D.S., Jr., K.K. Holmes, and G.A. Hill. 1976. *Cumitech 4, Laboratory diagnosis of gonorrhea.* Coordinating ed., S. Marcus and J.C. Sherris. American Society for Microbiology, Washington, D.C.
6. Dunne, W.M., Section 3, Aerobic bacteriology, 3.9.3, *In* L.S. Garcia (ed) *Clinical Microbiology Procedures Handbook*, 2010, American Society for Microbiology, Washington, D.C.
 |
| **Appendices** | WORKLABEL MEDIA FORM DEFINITIONBATTERY: GCSPEC MEDIA0 CHOC, MTMRECT MTMRS MTMTHR MTM |
| **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.
 |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Pat Ackerman | 1973 | Initial Version |
| 1.1 | Pat Ackerman | 11/1978 |  |
| 1.2 | Pat Ackerman | 07/20/2003 |  |
|  | 1.3 | Pat Ackerman | 12/10/2004 |  |  |  |
| 1.4 | Pat Ackerman | 08/05/2007 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Replaced API NH identification with Vitek NH. First voided urine specimens are only acceptable for men. Report UDET-NGON with overgrowth of yeast. |
| 1.5 | Jessica Craig | 05/28/2010 | Updated into online format. |
| 1.6 | Becky Carlson | 10/30/2013 | Revised online format.  |
| 2 | Becky Carlson | 4/16/2015 | Re-numbered from MC 420 for CMS load.  |
| 3 | Susan DeMeyere | 9/22/2017 | Removed Jembec media and workup. Added MALDI for identification. Update logo.  |
| **Archived by:** |  | **Archived Date:** |  |