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Detection of Chlamydia trachomatis and Neisseria gonorrhoeae

- Nucleic acid amplification tests (NAAT) have been shown to be more sensitive for detecting Chlamydia trachomatis and Neisseria gonorrhoeae than cultures.
- NAAT allow less invasive procedures for screening for CT/GC
- More sensitive than clinical diagnosis.
  - 70% of women are asymptomatic for C. trachomatis.
Common types of nucleic acid amplification tests for both CT and GC

- BD ProbeTec ET
- Roche Amplicor
- Roche COBAS
- Gen Probe Aptima
Selection of a test for the HPTN 035 study

- LCR was found to have false negative results with urine specimens containing small amounts of gel.
- LCR was taken off the market.
- ProbeTec was validated with urines containing varying concentrations of each of the gels.
- Urine specimens are less sensitive than cervical. Urines 83% sensitivity vs. cervical 96% sensitive.
- However urines do not require a speculum exam and will not have high concentrations of gel.
ProbeTec ET Strand Displacement Amplification (SDA)

- Advantages:
  - Test for CT/GC simultaneously
  - Less time to process than PCR
  - Sensitivity and Specificity comparable to other NAAT
Specimen Collection

• Patient should not have urinated for at least 1 hour.
• Collect 1st 15-20ml (Not mid-stream)
Specimen storage & Transportation, 2 options

- Urine that will be transported at room temperature
  - Add urine processing pouch (UPP) immediately after collection
  - Be careful in handling UPP. There is a potential of cross contamination
  - Always use a clean glove when handling and never set the pouch on the counter.
  - Urine is stable at room temp for 2 days.
Specimen storage & Transportation, 2 options

- Urine refrigerated at all times prior to testing
  - Do not need to add UPP at the time of collection (Add UPP at the lab 2 hours prior to processing)
  - Urine is stable for 4 days
Specimen Processing

- Prepare the heat block. Remove the lid to allow the temperature to equilibrate to 115°C. (Use the thermometer reading, not the digital reading)
- Label sample tubes.
- Add UPP to urine. It must be in contact for at least 2 hours before processing.
- Mix urine gently and transfer 4 ml into a sample tube
- Centrifuge at 2000g for 30 minutes
Specimen Processing

- Decant supernatant.
- *Critical step_* Must remove last drop with a flick of the wrist.
  - Inhibitors may be present in the urine.
  - 3 of 7 labs had 2X the rate of indeterminates due to poor decanting technique.
- Add 2 ml diluent. Cap and vortex.
- Specimen are ready for lysing or can be stored at RT for 6 hours or overnight at 2\(^\circ\)-8\(^\circ\)C.
QC Prep

- Must run a positive and negative control with each run and each new reagent kit lot number.
- Add diluent to the negative control first, then the positive (Less chance of cross contamination)
- Positive control **must** be in first well.
- The positive control detects reagent failure.
- The negative control detects reagent failure and environmental contamination.
- Enter the sample numbers into the computer and print out the Plate Layout Report
- Use the report to place the tubes in the lysing rack.
  - Check for clerical errors
External Quality Control

- For each new lot of reagent:
- Test 1 known positive and 1 negative patient sample from the previous lot.
- Record the results on an external QC report form. See handout.
Lysing Step

- After 30 minutes remove from the heat block and cool for at least 15 minutes.
- Tap the rack of tubes to shake the liquid out of the caps.
- The samples can now be stored for:
  - 6 hours at 18°-30°C
  - 5 days at 2°-8°C
  - 98 days or longer at -20°C to -80°C
Testing Procedure

- Use the Plate Layout Report to prepare the Priming and Amplification Plate.
- Green microwells (CT) in first column then the yellow (GC) wells in second column.
Testing Procedure

• Tap the rack of tubes again to shake the liquid out of the caps.
• Uncap the tubes. Be very careful when touching the caps to reduce the potential for cross contamination.
• Change gloves before opening the packet with the microwells.
Testing Procedure

- Using Program 2 on the pipettor, transfer 150ul into each well. (Program 3 if using AC)
- Do not draw up the sample from the bottom of the tube. Potential inhibitors are more concentrated at the bottom of the tube.
- Pay special attention to how you handle the pipette tips.
- *Collapse the tips before you put them over the wells.
- *Dispense liquid against the inside wall of the microwell
- Cover the microwell plate with the Priming Cover and incubate at RT for a minimum of 20 minutes and up to 6 hours.
Prepare Amplification Plate

- After the Priming plate has incubated at RT, remove the cover and place the plate on the Priming Heater.
- Immediately place the Amplification plate in the warming heater.
- Set the timer for 10 minutes. Critical time. Must be exact!
Prepare Amplification Plate

- Using Program 5 on the pipettor, transfer 100ul from column 1 of the Primer plate to column 1 of the Amplification plate.
  - When transferring allow the tips to touch the sides of the wells.
  - Dispense and mix.
  - Be careful when removing the tips from the wells. Potential for contamination
  - Carefully discard the tips.
Amplification

• Seal the Amplification plate
• Place the plate in the instrument and initiate the run.
• After 60 minutes the instrument will generate a report.
**Clean Up Procedure**

- Remove the Amp plate and discard in ziplock bag.
- Clean metal plate.
- Seal Priming Microwells and remove from heater.
- Cool.
- Remove the sealed microwells by holding the outer edges and lifting the well out of the unit.
- Discard the microwells in a ziplock bag.
- Clean the metal plate
- Rinse the plate with 1% sodium hypochlorite.
- Rinse with water. Dry.
Clean Up Procedure

- Clean the counter tops and exterior surfaces of the lysing heater, priming heater, and ProbeTec instrument with 1% sodium hypochlorite.
- Use several towels to complete the cleaning.
- Rinse with towels saturated with water.
- Wipe the handle only of the pipettor with 1% sodium hypochlorite and rinse.
Interpretation of Results

- QC results
  - CT/GC positive control MOTA > 2000
  - Negative control MOTA < 2000

- Specimen Results
  - Negative result MOTA < 2,000
  - Positive result MOTA > 2,000
  - Low Positive Results MOTA 2000 to 10,000
  - With Low Positive Results there is a decreased likelihood of being a true positive.
  - Repeat test on lysed sample that has been frozen.
Amplification Controls

- Optional test for detecting inhibitors of amplification
- AC<1,000 and CT or GC <2,000=Indeterminate Results.
- Range from 1% to 16% of urine samples.
- In a study of 1331 urine samples
  - 13% indeterminates
  - 0.07% were GC positive
  - 0.5% CT positive
Quality Assurance

• Compare print out to patient log book.
• Review the report print out daily for clusters of positive.
• Clusters can be an indication of cross-contamination.
• Re-test clusters.
• Calculate the % positive monthly for each clinic and overall.
• Chart the percentages.
Reports

• File all original print out of the results (source documents).
• Patient report forms must indicate the method of testing. (SDA)
• The reports must be reviewed and signed by the lab manager or qualified designated person.
Monitoring for the presence of DNA Contamination

Monthly, monitor the following areas for DNA contamination:

Area 1: Lysing Heater, Lysing Rack, Priming and warming heater, and Black microwell trays

Area 2: Pipettor handle, Instrument keys and keyboard, Instrument door release, and inside carrier.

Area 3: Centrifuge drum and Work benches
Procedure for Monitoring DNA Contamination

1. Label 3 sample tube containing 2 ml of diluent.
2. Dip a sterile swab into the diluent and wipe the surfaces with a broad sweeping motion of Area 1.
3. Express the swab in the diluent tube.
4. Recap the tube and vortex for 5 seconds.
5. Repeat for areas 2 and 3.
6. Tubes are ready for lysing.
7. Record the MOTA score on the QC monitoring for DNA contamination log form. (See handout)
8. If an area is positive, clean all surfaces within the area and repeat the test.
Storage of Specimens

- Freeze all lysed samples for potential repeat testing.
- Save all positive samples for QC and training purposes.
- Temporary and permanent storage system.
Limitations

- Blood in concentrations > 5% was shown to cause indeterminate (inhibitory) results in urine and swab specimens (with Amplification Controls) and false negative results in urine specimens (with and without AC).
- BD ProbeTEc cannot be used to assess therapeutic success or failure. Nucleic acids from CT or GC may persist after therapy.
Limitations

- ProbeTec results are qualitative. No correlation can be drawn between the magnitude of MOTA score and the number of cells in an infected sample.
- Testing urine specimens from female patients as the sole test for identifying CT or GC infections may miss infected individuals (17% of females with CT positive cultures and 13.8% of females with GC positive cultures had negative results when urine only was tested) with ProbeTec.