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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 115^0C. (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°-8^oC.

QC Prep

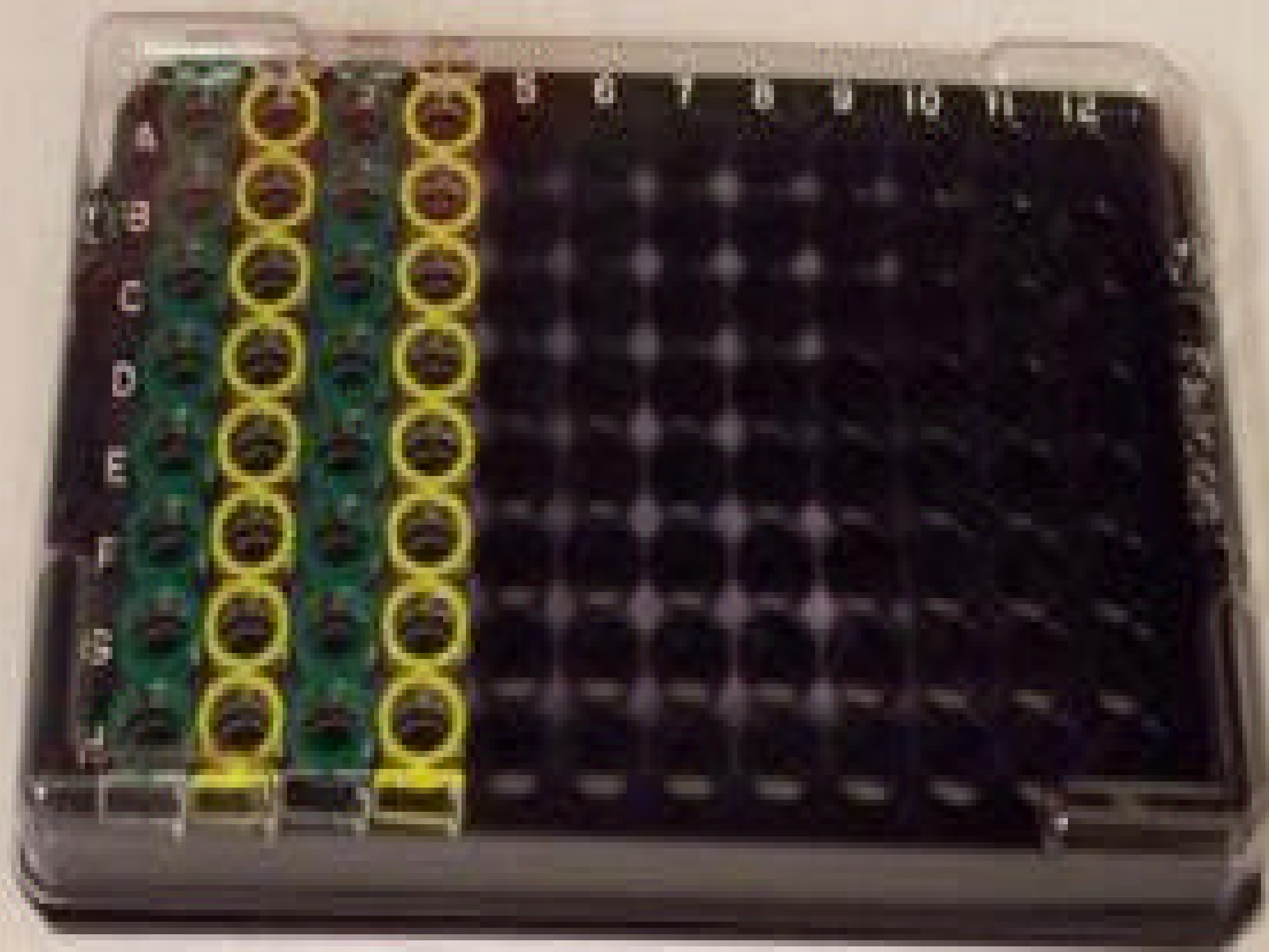
- Must run a positive and negative control with each run and each new reagent kit lot number.
- Add diluent to the negative control first, then the positive (Less chance of cross contamination)
- Positive control **must** be in first well.
- The positive control detects reagent failure.
- The negative 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

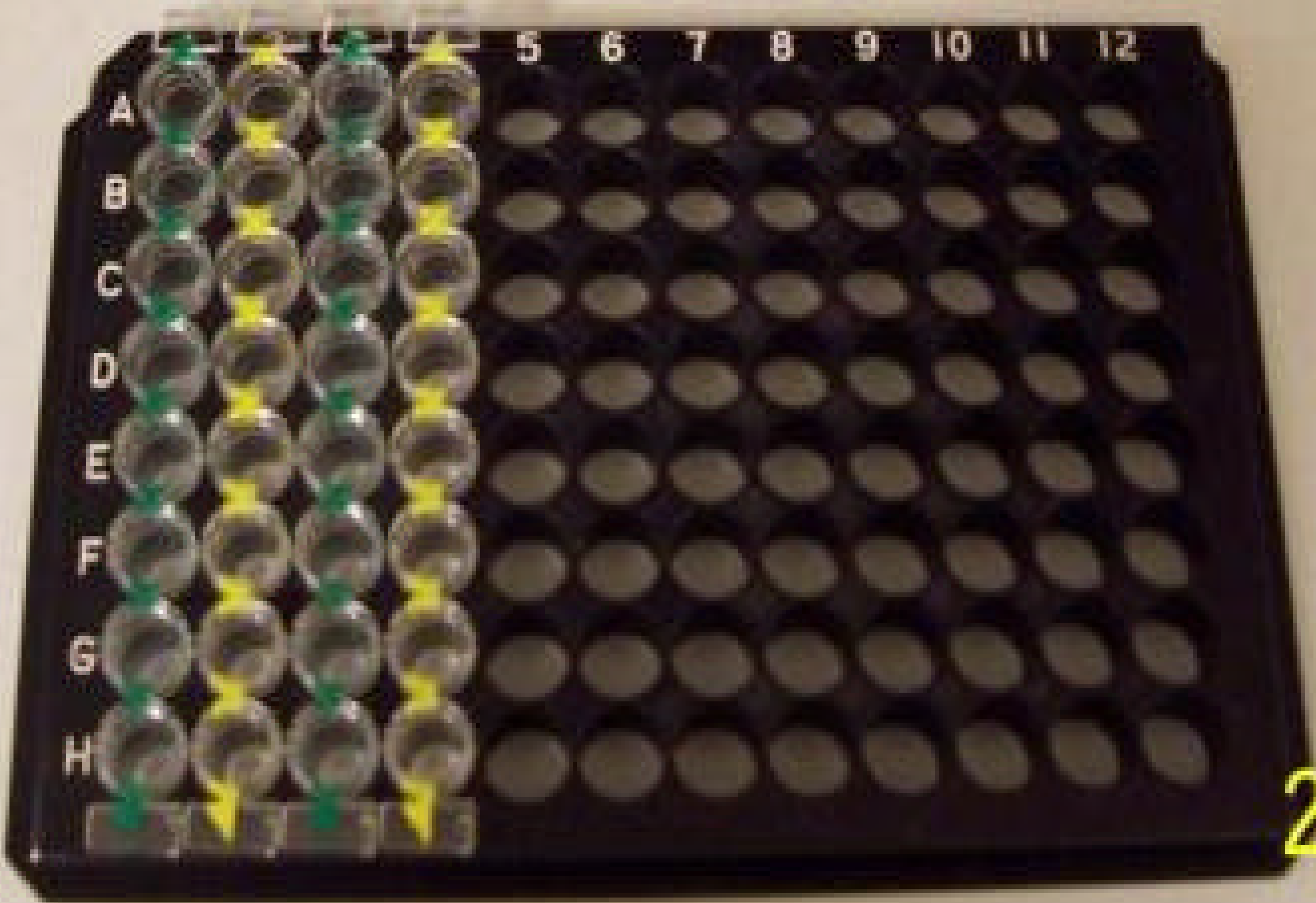
- For each new lot of reagent:
- Test 1 known positive and 1 negative patient sample from the previous lot.
- Record the results on an external QC report form. See handout.

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^oC
- 5 days at 2°-8^oC
- 98 days or longer at -20^oC to -80^oC

- 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.

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- 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.

- 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 $\geq$2000
- Negative control MOTA $<$ 2000
- **Specimen Results**
- Negative result MOTA$<$2,000
- Positive result MOTA$\geq$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.

- 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:

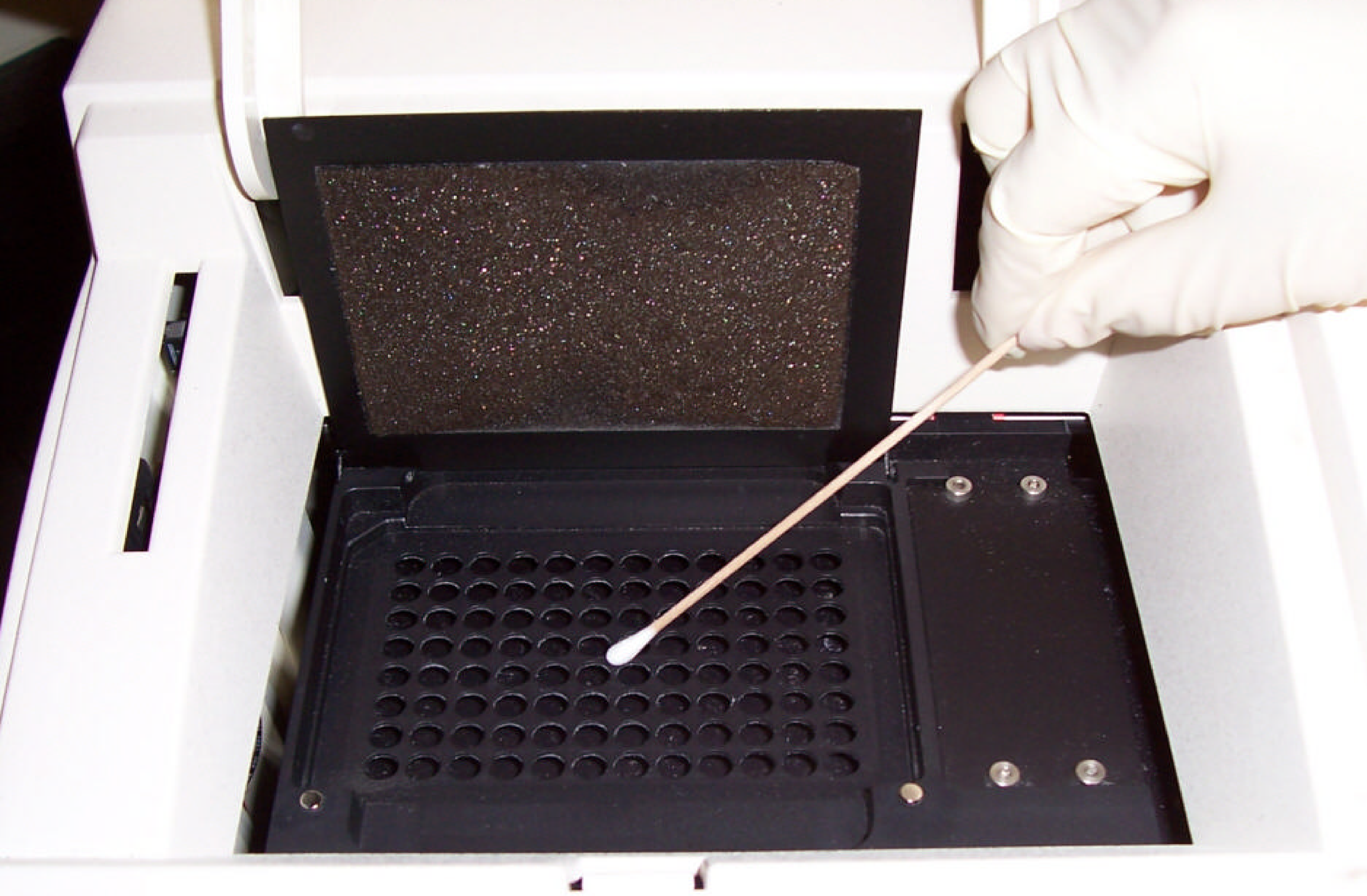
Area 1:Lysing Heater,Lysing Rack, Priming and warming heater, and Black microwell trays

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.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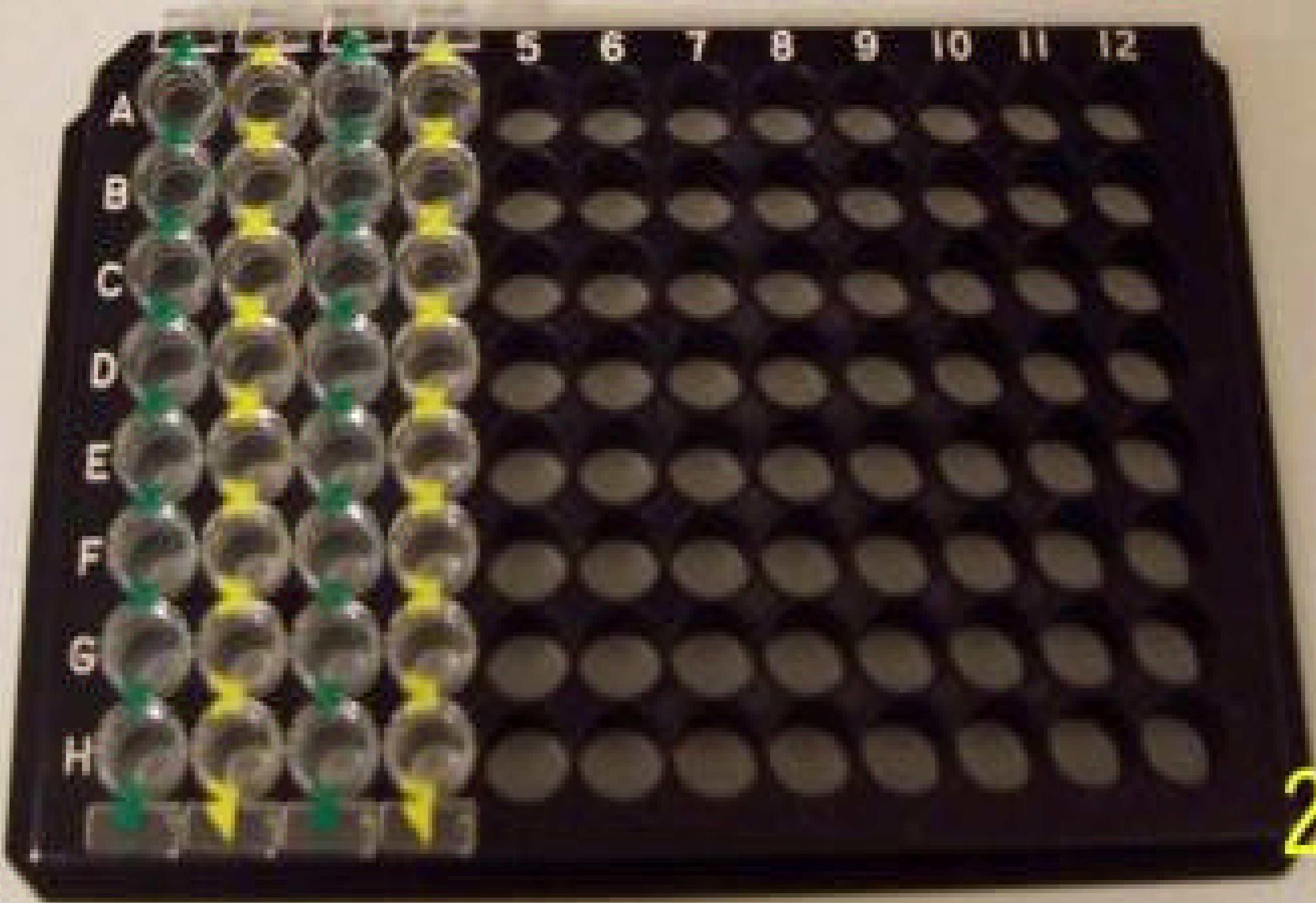
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Storage of Specimens

- 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.