



# Clinical Laboratory Department POLICY AND PROCEDURE

POLICY NUMBER: 1226  
VERSION: 4

## **SUBJECT: Modified Diamond's Media**

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### Principle and Description

Modified Diamond's Media is a fluid (semi-solid) tubed media designed to enhance the transport and growth of *Trichomonas vaginalis*. It is supplied by Hardy Diagnostics Media, catalog #K02. Storage of the uninoculated media is at 2-8°C; shelf life is approximately 6 months to a year. The media is inoculated at patient bedside (clinic) with a vaginal swab, which is broken off in the media. The media is then transported to the laboratory where it is incubated, tightly capped. Incubation temperature must be between 34° and 36°C. Temperatures warmer or cooler than that range can depress the motility of the organism.

### Procedure

1. Inoculated Diamond's media for the Wet Mount test are received into the laboratory daily. Wet Mounts cannot be resulted STAT.
2. Incubate the patient specimens after receipt in the 35°C incubator without CO<sub>2</sub> or in the heat block.
3. The day after the wet mounts are collected, generate a work list through the computer system.
4. Read the specimens microscopically at High Power, using the worklist to organize the samples and make sure none are missing.

### Recording and Reporting

1. 24 hours after inoculation, read each wet mount. Record whether yeast is present and quantitate (few to 4+). Record whether Trichomonads are present and quantitate (few to 4+). Record if the sample is negative for both yeast and/or Trichomonas. Use the Microbiology Coded Response Sheet. Do not document presence of WBC's, clue cells or red cells. If there is an unusual organism or structure present, verify your observation by having a second opinion, preferably by a supervisor.

## Quality Assurance and QC Procedures

Each lot/shipment of Modified Diamond's media shall have end-user QC performed. Two tubes shall be taken from each lot of the shipment, and set aside for testing. The QC organisms and inoculation protocol is as follows:

Stock #49 *Trichomonas vaginalis* SJCR 66 or wild strain  
Stock #53 *Candida albicans* ATCC 10231 **OR**  
Stock #1 *Escherichia coli* ATCC 25922

1. Tube A: Inoculate a fresh growth of *Trichomonas vaginalis* SJCR 66 or wild strain using a glass or plastic pipette directly into the test media. Incubate at 35°C for 24-96 hrs. Examine for presence of growth AND observe microscopically for motile, twitching organisms.
2. Tube B: Prepare a 0.5 MacFarland dilution of fresh *C. albicans* in saline. Make a 1:10 dilution of the suspension. Inoculate 10 µL of the dilution into the Diamond's media. Incubate at 35°C for 24 hrs. Examine visually for signs of growth. **OR**
3. Tube B: Prepare a 0.5 MacFarland dilution of fresh *E. coli* in saline. Make a 1:10 dilution of the suspension. Inoculate 10 µL of the dilution into the Diamond's media. Incubate at 35°C for 24 hrs. Examine visually for signs of growth.
4. Document all findings on the Media Quality Control Worksheet. If there is any discrepant result, it must be resolved before the media is used for patient testing.
5. Each day Wet mounts are read, read and record presence of motile *Trichomonas* from the working stock of *Trichomonas vaginalis*, #49 (or wild strain). Record on the daily QC log.

Semi-annual test validation must be done for wet mounts since we do not have a proficiency test program in place. Choose a "not so random" wet mount, record the results, and have another CLS read and record the second reading on the worksheet located on the bulletin board in the micro lab.

### Limitations on Media QC results:

1. The stock organism *Trichomonas vaginalis* SJCR 66, has been shown to be prone to contamination, especially by yeast and streptococci. Extreme care must be used in transferring the organism from one tube of Diamond's media to a fresh tube.
2. *Candida albicans* ATCC 10231 appears to thrive in Diamond's media. This anomaly was presented to the technical support team at Hardy media. The QC specialists at Hardy were unable to duplicate the heavy growth of *C. albicans* in their test lab that was witnessed at this lab. However, the User Quality Control section of the Diamond's media procedure states "Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction". That statement has been satisfied with the performance of the *Trichomonas vaginalis* SJCR 66 or wild strain (positive) and the *Escherichia coli* ATCC 25922 (negative).
3. Suppression of *Candida albicans* or other yeast species is not a clinical issue in this lab, as all specimens are read at approximately 24 hours, and are not tampered with prior to the one reading. This is a departure from the published Hardy recommendation of reading the specimens at 24, 48, and 96 hours.

Consultation with Hardy technical service resulted in a modification of the written procedure to reading at 24 or 48 hours only. In addition, an in-house study was performed, reading approximately 1,000 wet mounts. Only two patient specimens displayed motile Trichomonads at 48 hours and not at 24 hours.

4. Trichomonads prefer an anaerobic environment. Keep the cap tightly closed, and transfer the organism from the lower half of the tube when subculturing.

#### QC Organism maintenance (*Trichomonas vaginalis* SJCR 66)

1. The organism arrives in a special incubation pouch called an In-pouch. Remove a sample of the fluid from the pouch using a glass pipet (using care to not break the glass tip in the pouch).
2. Inoculate a tube of Diamond's media; allow to incubate in a 35°C heat block for 24 hrs.
3. Examine for presence of motile trichomonads.
4. Repeat this procedure for 3 days total, removing a portion of the original pouch and inoculating a fresh Diamond's media tube. Label each tube clearly as to contents and DATE.
5. Examine each tube (microscopically) daily until a strong positive culture is observed, with many trichomonads present with good to excellent motility.
6. Use this stock culture as your beginning stock. From this tube(s), inoculate a fresh tube of Diamond's media **each day**.
7. Read a well-established positive QC sub for your daily documentation.
8. Be sure to verify that a strongly positive growth is achieved in at least one of your subs before discarding "old" QC subs. It is estimated that 2 to 3 tubes of QC material will be incubating at any one time.
9. Since the In-pouch is very sensitive, expensive, and difficult to keep alive, it is alternatively acceptable to keep an in-house strain alive by the same transfer process and used for QC purposes.

#### References:

HUGO (Hardy Users Group Observer), vs 6. Modified Diamond's Media Package Insert. 05/12/2006.

Hardy Diagnostics Technical Service. Natalie, phone discussion 09/24/08

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<b>Approved By:</b> Brian Yee (PHYS SPEC PATHOLOGY)	
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<b>Revised:</b>	4/27/09 delete 2 <sup>nd</sup> readings, 2 QC only needed at intake, add limitations to QC results, 5/21/10 jh-included using wild strain for QC. 6/18/13 jh typos and clarity only, 3/21/17jh changed approver to Dr. Yee
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