

QUALITY CONTROL

I. INTRODUCTION:

QC programs ensure that the information generated by the laboratory is accurate, reliable, and reproducible. This is accomplished by assessing the quality of the specimens; monitoring the performance of test procedures, reagents, media, instruments, and personnel; reviewing test results; and documenting the validity of the test method.

II. SPECIMEN COLLECTION AND TRANSPORT:

- A. Do not test unacceptable specimens unless they cannot be re-collected. When unacceptable specimens must be processed, include a disclaimer on the report indicating that the specimen was not acceptable and is being processed at the request of the physician.
- B. Specimens to be rejected after notifying supervisor and physician in charge of patient.
 - 1. Swabs used for multiple procedures
 - 2. Cerebro spinal fluids with less than 0.3 cc for Cryptococcus antigen
 - 3. Hemolized or Lipemic specimens for fungus serology

III. RECORDS:

- A. Laboratory personnel are responsible for recording quality control data on the appropriate forms provided by the supervisor.
- B. The supervisor is responsible for monthly periodic review of the quality control data and documentation of the review (initial and date).
- C. The supervisor is responsible for maintaining quality control records in an organized manner available for inspection.
- D. All quality control records will be retained for a period of at least two years.

IV. PROCEDURE MANUALS:

- A. Procedural manuals will be available at all times in the laboratory.
- B. Instructions for every procedure routinely performed in the laboratory are included in the manuals.
- C. The procedure manuals will be reviewed, dated and initialed by the director of the laboratory each year.
- D. Procedure modifications or new protocols will be dated and initialed by the director when added to the procedure manual.
- E. Laboratory procedures used routinely will be those that have been published in reputable microbiological books, manuals, or journals. Procedures that have not been described in publications may be used, provided that the necessary experimental control studies have been performed in a competent manner and a description of the procedure is expected to be published.
- F. Procedures in the manual will be referenced to a published source.

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V. STERILITY DETERMINATION PROCEDURE:

A. Media (prepared in-house)

1. At random, select five percent of the units prepared in each lot of medium to be tested.
2. Incubate half of the units selected at 35 °C for 3 days. Incubate the other units at 25 °C for 3 days.
3. Inspect the units daily for contamination.
4. If 5% or less of the total units sampled are contaminated, the lot is acceptable for use. The sampled units used for testing are discarded. Results are recorded on the appropriate form, dated and initialed.
5. If more than 5% of the total units sampled are contaminated, the lot is resampled. If the resampled units again show greater than 5% contamination, the entire lot is assumed to be contaminated and is discarded.

VI. EQUIPMENT:

A. General

1. Each item of equipment in the laboratory will meet with the manufacturer's claims and specifications.
2. Monitoring procedures performed by the laboratory staff will assure the constant accuracy and precision necessary for quality laboratory performance.
3. Equipment failure or malfunction will be reported to the supervisor as soon as it is detected. The supervisor is responsible for evaluating and correcting the failure or malfunction. A deficiency documentation form should be attached to the equipment daily QC record.
4. Laboratory personnel will be thoroughly familiar with the operation of each piece of equipment before using it.
5. Each piece of electrical equipment will be inspected by the Biomedical Engineering Department. Records are kept by the Biomedical Engineering Department. After the safety inspection a dated inspection sticker is placed on the piece of equipment.

B. Glassware

1. All chipped, damaged or etched glassware will be discarded to prevent accidents.
2. Sterilized glassware will not be stored more than 3 weeks before use.
3. All clean and sterilized glassware will be covered with aluminum foil and labelled with the date of sterilization.
4. Glassware will be washed with Sparkleen or other detergent free cleaners.

VII. MEDIA, REAGENTS, STAINS, BIOCHEMICAL REACTION, AND DRUG SUSCEPTIBILITY TESTING:

A. General

1. Storage and labelling procedures - All media, solutions and reagents will be labelled with the item name, lot number and expiration date. All media, solutions and reagents will be labelled with the date of first in use.

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VII. MEDIA, REAGENTS, STAINS, BIOCHEMICAL REACTION, AND DRUG SUSCEPTIBILITY TESTING:

2. Sterility checks
 - a. Media and solutions will not be made available for use until sterility checks are performed. Contaminated lots of media and solutions will be discarded. (See sterility check procedure).
 - b. Media or solutions prepared or purchased by the Clinical Mycology laboratory will be checked for sterility by its laboratory staff. Results will be kept in the Mycology laboratory.
3. Inspection of media - All media is to be checked for proper color, cracks, dryness and contamination on arrival. A record of this inspection is to be added to the Quality Control Sheets.
4. Performance evaluation
 - a. A performance evaluation is done on new lots of media, and on reagents and solutions used in biochemical test procedures and staining procedures. For commercially prepared media, those tested in NCCLS M22-A are exempted from QC testing by the user. Only a visual check will be performed along with a record of lot number and expiration date will be kept.
 - b. Performance records of the media by the manufacturer will be provided with the shipment of the medium. Organisms are selected to demonstrate the expected performance of the item tested. Some items require only the organism to demonstrate adequate performance. Some items require two or three organisms to demonstrate positive and negative reactions.
 - c. All QC organisms are to be kept frozen at -70°C in potato dextrose slants for indefinite period of time. A set growing at room temperature should be kept and resubcultured every three months. Fresh isolates should be taken from the -70°C freezer every year. All media will be evaluated for performance except for those listed in NCCLS M22-A. Performance evaluation should be done prior to use, however in some instances, performance is evaluated concurrently with the use of the media. For a list of expected reactions, refer to Table 6. The laboratory procedure manuals contain a media section which includes ingredients, conditions, shelf life, sterility and performance for each medium used in the laboratory. Purchase or preparation of media will be limited to a 6 month supply.
5. Stains - All staining solutions will be labelled with the name of the solution and the lot number, expiration date and storage conditions. Commercially prepared stains will be dated on the container when received and again when first opened for use. Stains prepared in the laboratory will also be labelled with the preparation date and the name of the person who prepared the stain. All staining procedures will be checked for performance using positive and negative control slides. Results will be recorded, initialed and dated, on the appropriate form. The laboratory procedure manuals contain instructions for stain preparation, staining procedures, storage conditions, and criteria for performance evaluation. Staining solutions will be discarded when the performance is unsatisfactory. For a list of expected reactions, refer to Table 6.

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6. Reagents and Solutions - All reagents and solutions used in the laboratory will be labelled with the name, lot number, expiration date and storage conditions. Commercially prepared solutions and reagents will be dated on the container when received and again when first opened for use. Solutions and reagents prepared in the laboratory will also be labelled with the preparation date and the name of the person who prepared the solution. The laboratory procedure manuals contain instructions for reagent and solution preparation, storage conditions, shelf life and criteria for performance evaluation when appropriate. Purchase or preparation of reagents and solutions will be limited to a 6 month supply. For a list of expected reactions, refer to Table 6.
7. Biochemical Tests
 - a. All biochemical tests will be performed according to the instructions outlined in the procedure manuals. The procedure manuals designate which controls to use and the expected results. Results will be recorded, dated and initialed on the appropriate forms. Lot numbers or reagents are recorded where applicable.
 - b. Unsatisfactory results will be immediately brought to the attention of the supervisor. No results will be used in the identification of organisms unless the controls show expected results. For a list of expected reactions, refer to Table 6.
8. Drug Susceptibility Tests
 - a. The drug susceptibility medium is evaluated for sterility and performance concurrently with the performance of the test. Results of the drug susceptibility tests will not be reported unless the controls yield the expected results.
 - b. Instructions for performing and evaluating drug susceptibility tests are detailed in the procedure manuals.
 - c. All results will be recorded, initialed and dated on the appropriate form. For a list of expected reactions, refer to Table 6I.

VIII. PROFICIENCY TESTING PROGRAM:

- A. In order to keep accreditation the laboratory will participate in an external proficiency program reflecting the specialty of the laboratory (Mycology) and level of expertise.
- B. In order to keep impartiality with the specimens submitted for proficiency all specimens are to be treated as a patient.
 1. Proficiency specimens should be processed within one day of arrival.
 2. Specimens should be accessioned in the computer system and labels to be generated using the proper charge account or unit history number designed for proficiency testing.
 3. A direct mount is to be prepared for all specimens if required.
 4. Cultures will be processed and worked up following section guidelines including preliminary results in the computer system (LIS).
 5. All final reports will be reviewed and signed by the supervisor and director.
 6. The 1988 Clinical Laboratory Improvement Act proposes that all laboratories be required to maintain an average score of 80% on proficiency test.

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VIII. PROFICIENCY TESTING PROGRAM:

- C. Testing of Analytes Not Covered by CAP or Other Proficiency Service - Internal proficiency programs are used to monitor various aspects of laboratory work which is not covered by external proficiency programs such as fungal immunodiffusions and fungus susceptibility testing. Records will be kept in the laboratory of such testing. Testing will include but not be limited to the following.
1. Reprocessing of test by different personnel for fungal immunodiffusion that will include a positive and negative result.
 2. Specimens to be sent to a different laboratory for comparison for susceptibility testing.
 3. Documentation will be recorded and attached to Quality Assurance Workbook.
- D. Competency Testing of Personnel - Internal testing of personnel is used to monitor various aspects of laboratory work which may not be covered by proficiency testing such as the many details involved in the identification of fungi, from the isolation of the organism in the plates to the final report in the computer. All testing personnel should maintain their competency to perform test procedures, report test results promptly, accurately, and proficiently.
1. Personnel will be tested and documented for performance at least semi-annually during the first year; thereafter, evaluations must be performed at least annually unless test methodology or instrumentation changes. If there are any changes in methodology or instrumentation prior to reporting test results, performance should be re-evaluated.
 2. Competency revalidation activities should be used for documentation. records should be maintained by QA coordinator and supervisor.

IX. REFERENCES:

1. Isenberg, HD: Clinical Microbiology Procedures Handbook. Washington, DC, ASM Press, 1992.
2. McGinnis, MR: Laboratory Handbook of Medical Mycology, New York, Academic Press, 1980.
3. NCCLS: Quality Assurance for Commercially Prepared Microbiological Culture Media: Approved Standard, NCCLS Document M 22-A, Villanova, PA, NCCLS, 1990.
4. NCCLS: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast; Proposed Standard, NCCLS Document M27-P, Villanova, PA, NCCLS, 1992.

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Table 1
Culture Collection Stock Organism for Quality Control of Reagents and Media

UTMB 125	<i>Saccharomyces cerevisiae</i>	(ATCC 9763)
UTMB 126	<i>Paecilomyces variotii</i>	(ATCC 36257)
UTMB 160	<i>Trichophyton tonsurans</i>	(Duke 914)
UTMB 843	<i>Nocardia asteroides</i>	(CDC B-2965) "Freddie"
UTMB 3515	<i>Actinomadura madurae</i>	
UTMB 417	<i>Trichophyton rubrum</i>	(LAIR 63)
UTMB 377	<i>Trichophyton equinum</i>	
UTMB 422	<i>Trichophyton mentagrophytes</i>	(LAIR 100)
UTMB 535	<i>Nocardia brasiliensis</i>	(CDC-4243)
UTMB 698	<i>Aspergillus flavus</i>	(CDC B-15)
UTMB 785	<i>Aspergillus fumigatus</i>	
UTMB 220	<i>Microsporium gypseum</i>	(Duke 136)
UTMB 3523	<i>Blastoschizomyces capitatus</i>	(ATCC 10663)
UTMB 3524	<i>Cryptococcus laurentii</i>	(ATCC 12803)
UTMB 3525	<i>Candida tropicalis</i>	(ATCC 13803)
UTMB 3526	<i>Trichosporon beigeli</i>	(CAP)
UTMB 3527	<i>Cryptococcus albidus</i>	(ATCC 34140)
UTMB 3528	<i>Candida albicans</i>	(ATCC 14053, UTMB 1)
UTMB 3529	<i>Torloopsis glabrata</i>	(ATCC 2001)
UTMB 3530	<i>Cryptococcus laurentii</i>	(ATCC 76483)
UTMB 3531	<i>Candida lypolytica</i>	(ATCC 9773)
UTMB 3532	<i>Cryptococcus neoformans</i>	(ATCC 76484)
UTMB 3533	<i>Cryptococcus neoformans</i>	(ATCC 32045)
UTMB 420	<i>Streptomyces griseus</i>	(CDC-N237)
UTMB 3502	<i>Candida albicans</i>	(ATCC 90028)
UTMB 3536	<i>Coccidioides immitis</i>	(ATCC 28868)
UTMB 3537	<i>Histoplasma capsulatum</i>	(ATCC 38904)
UTMB 3538	<i>Blastomyces dermatitidis</i>	(ATCC 60916)

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Table 2
QC of Media, Reagents, and Test Expected Reactions

Vitek Assumitation BC Cards	Identification Within	
1) <i>Cryptococcus neoformans</i>	ATCC 76484	98-99% probability
2) <i>Candida lipolytica</i>	ATCC 9773	98-99% probability

Run with each new lot number.

API Assimilation

1) <i>Trichosporon beigeli</i>	2744771
2) <i>Torulopsis glabrata</i>	2000040

Run once with each new lot number.

Germ Tube

1) <i>Candida albicans</i>	Positive control - germ tube
2) <i>Candida tropicalis</i>	Negative control - no germ tube

Run each time germ tubes are set up.

Potato Flake Agar

1) <i>Candida albicans</i>	growth
2) Uninoculated plate	no growth

Run with each new lot number is received.

Urea Agar Slants

1) <i>Cryptococcus neoformans</i>	pink color	positive reaction
2) <i>Candida albicans</i>	no color or yellow color	negative reaction
3) Uninoculated	no color or yellow color	negative reaction

Run with each new lot number.

Potato Dextrose Agar Slants

1) <i>Candida albicans</i>	growth
2) Uninoculated slant	no growth

Run with each new lot number.

Potato Dextrose Plates

1) <i>Candida albicans</i>	growth
2) Uninoculated plate	no growth

Run with each new lot number.

Cornmeal Agar w/Tween 80 plate

- | | |
|----------------------------|-----------|
| 1) <i>Candida albicans</i> | growth |
| 2) Uninoculated plate | no growth |

Run with each new lot number.

Saboraud Dextrose Broth

- | | |
|-----------------------------------|-----------|
| 1) <i>Cryptococcus neoformans</i> | growth |
| 2) Uninoculated broth | no growth |

Inhibitory Mould Agar (IMA)

- | | |
|----------------------------|-----------|
| 1) <i>Candida albicans</i> | growth |
| 2) <i>Escherichia coli</i> | no growth |
| 3) Uninoculated plate | no growth |

Run with each new lot number.

Brain Heart Infusion Agar Plates

- | | |
|-----------------------------------|-----------|
| 1) <i>Cryptococcus neoformans</i> | growth |
| 2) Uninoculated plate | no growth |

Run with each new lot number when the medium is prepared in house; otherwise, the medium is exempt for QC by user.

Brain Heart Infusion Agar Plates with 10% Sheep Blood and Chloramphenicol and Gentamycin

- | | |
|----------------------------|-----------|
| 1) <i>Candida albicans</i> | growth |
| 2) <i>Escherichia coli</i> | no growth |
| 3) Uninoculated plate | no growth |

Run with each new lot number when the medium is prepared in house; otherwise, the medium is exempt for QC by user.

Mycosel Slants

- | | |
|---------------------------------------|-------------------|
| 1) <i>Trichophyton mentagrophytes</i> | growth |
| 2) <i>Aspergillus flavus</i> | poor to no growth |
| 3) Uninoculated slant | no growth |

Run with each new lot number when the medium is prepared in house; otherwise, the medium is exempt for QC by user.

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Table 2 - cont'd

Yeast Conversion - BHI with 10% sheep blood slant

- 1) *Histoplasma capsulatum* growth/yeast conversion
- 2) uninoculated slant no growth

Run with each new lot number

Hair Perforation

- 1) *Trichophyton mentagrophytes* perforation positive control
- 2) *Trichophyton rubrum* no perforation negative control

Run each time test is set up.

Trichophyton Agar #1, #2, #3, #4

- 1) *Trichophyton mentagrophytes* good growth on tubes #1,2,3,4
- 2) *Trichophyton tonsurans* good growth on tubes #3,4
poor growth on tubes #1,2
- 3) *Trichophyton verrucosum* good growth on tubes #2,3
poor growth on tubes #1,4

Run with each new lot number.

Rice Grains - Rice Grain Medium

- 1) *Trichophyton mentagrophytes* good growth
- 2) *Microsporum audouinii* no growth brown color
- 3) *Microsporum canis* good growth bright yellow color

Run with each new lot number.

Urea Hydrolysis (Dermatophytes) - Urea-agar

- 1) *Trichophyton mentagrophytes* pink color positive reaction
- 2) *Trichophyton rubrum* no color negative reaction
- 3) Uninoculated

Run with each new lot number.

Malt Agar

- 1) *Candida albicans* growth
- 2) Uninoculated plates no growth

Run with each new lot number.

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Table 2 - cont'd

Czapek Agar

- 1) *Aspergillus flavus* growth
- 2) Uninoculated plate no growth

Run with each new lot number.

Actinomycete Hydrolysis Plates

- 1) Casein
 - a) *Nocardia asteroides* growth no clearing of agar negative reaction
 - b) *Streptomyces griseus* growth clearing of agar positive reaction
- 2) Tyrosine
 - a) *Nocardia asteroides* growth no clearing of agar negative reaction
 - b) *Streptomyces griseus* growth clearing of agar positive reaction
- 3) Xanthine
 - a) *Nocardia asteroides* growth no clearing of agar negative reaction
 - b) *Streptomyces griseus* growth clearing of agar positive reaction
- 4) Uninoculated Tri-plate No growth

Run with each new lot number.

Lysozyme Test

- 1) Lysozyme Broth
 - a) *Nocardia brasiliensis* growth
 - b) *Streptomyces griseus* no growth
- 2) Basal Broth
 - a) *Nocardia brasiliensis* growth
 - b) *Streptomyces griseus* no growth

Run each time test is set up.

Calcofluor - Direct Preparation Stain

Candida albicans spp. bright blue or apple green fluorescens to be run each week

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Table 2 - cont'd

Antifungal Susceptibility Testing

Yeast Susceptibility Testing

Candida albicans UTMB 3502

1) Amphotericin B	0.25 - 1.0 µg/ml
2) Clotrimazole	0.22 - 14.00 µg/ml
3) 5-Fluorocytosine	1.0 - 4.0 µg/ml
4) Fluconazole	≤ 0.125 - 0.9 µg/ml
5) Itraconazole	0.3 - 0.5 µg/ml
6) Ketoconazole	≤ 0.03 - 0.125 µg/ml
7) Nystatin	0.57 - 4.6 µg/ml

Run each time a drug is setup.

DNA Identification Probes

1) Test: *Histoplasma capsulatum* Culture Identification Kit

- a) *H. capsulatum* (ATCC 38904) Positive control expected result = > 50,000 RLULeader I)
- b) *Blastomyces dermatitidis* (ATCC 60916) Negative control expected result = < 20,000 RLULeader I)

2) Test: *Blastomyces dermatitidis* Culture Identification Kit

- a) *B. dermatitidis* (ATCC 60916) Positive control expected result = > 50,000 RLULeader I)
- b) *H. capsulatum* (ATCC 38904) Negative control expected result = < 20,000 RLULeader I)

3) Test: *Coccidioides immitis* Culture Identification Test

- a) *C. immitis* (ATCC 28868) Positive control expected result = > 50,000 RLULeader I)
- b) *B. dermatitidis* (ATCC 60916) Negative control expected result = < 20,000 RLULeader I)

Run each time test is set up.