TRAINING UPDATE

Lab Location:	Core Lab	**Date Distributed:**	8/8/2022
Department:	Microbiology	**Due Date:**	8/22/2022

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Technical SOP: Resulting Microbiology Direct Exams (AHC.M28 v.4) and Wet Prep (AHC.M23 v.8).

Description of change(s):

The wet prep keyboard has been updated to allow reporting to match SOP.

The Wet Prep SOP, section 10.1, states; "Report the presence or absence of Trichomonas, yeast and clue cells". Up until the update, we were not able to report the absence of those organism individually. The keyboard update now allows for reporting "Yeast not seen", "Clue cells not seen" and "Trichomonas not seen". We will now report the absence, in addition to presence, of those organisms, as necessary per the SOP.

For details on the updated keyboard, refer to the SOP "Resulting Direct Microbiology Exams". See highlighted sections on attached copy of SOP for changes.

This revised SOP will be implemented on August 23, 2022

Document your compliance with this training update by taking the quiz in the MTS system.

AHC.M23 Wet Prep

Copy of version 8.0 (in review)

Effective Date 8/23/2022

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Organization Adventist HealthCare

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	7/8/2022	7.0	Nicolas Cacciabeve	
Approval	Microbiology Lab Director	7/7/2022	7.0	Ronald Master	
Approval	Lab Director	7/8/2020	6.0	Nicolas Cacciabeve	
Approval	Micro Director approval	7/7/2020	6.0	Ronald Master	
Approval	QA approval	7/6/2020	6.0	Leslie Barrett	
Approval Captured outside MediaLab	Lab Director	7/16/2018	5.0	Nicolas Cacciabeve	Recorded on 11/21/2018 by Leslie Barrett when document added to MediaLab
Periodic review Captured outside MediaLab	Designated Reviewer	7/16/2018	5.0	Nicolas Cacciabeve	Recorded on 11/21/2018 by Leslie Barrett when document added to MediaLab

Approvals and periodic reviews that occurred before this document was added to the MediaLab Document Control system may not be listed.

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
7.0	Approved and Current	Major revision	7/7/2022	7/8/2022	Indefinite
6.0	Retired	Major revision	7/6/2020	7/8/2020	7/8/2022
5.0	Retired	First version in Document Control	11/21/2018	8/7/2018	7/8/2020

Technical SOP

Title	**Wet Prep**		
Prepared by	Ron Master	Date:	8/25/2009
Owner	Ron Master	Date:	8/25/2009

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Wet Prep	N/A	WETP

Synonyms/Abbreviations
N/A

Department
Microbiology

2. ANALYTICAL PRINCIPLE

Yeast and *Trichomonas* sp. can be found in urine or vaginal discharges. *Trichomonas* move by a progressive undulating, whipping of flagella and pseudopodial movement. Outside the body *Trichomonas* rapidly succumbs at temperatures higher than 40°C; therefore it is imperative to examine specimens requested for *Trichomonas* immediately upon arrival in the Laboratory. Yeast and clue cells can also be detected from vaginal discharge.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Specimen should be obtained on a swab and submitted in a tube containing 0.5 mL 0.85-0.9% saline (0.85-0.9% sodium chloride). A red top vacutainer tube (without additives) may be used. Deliver to Laboratory immediately.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type	**-Preferred**	Vaginal discharges.
	-Other Acceptable	None

Criteria	
Collection Container	Swab in tube or a red-top vacutainer (without additives) containing 0.5 mL 0.85-0.9% saline
Volume - Optimum **- Minimum**	Swab in 0.5mL 0.85-0.9% saline Swab in 0.5mL 0.85-0.9% saline
Transport Container and Temperature	Collection container at room temperature
Stability & Storage Requirements	Room Temperature: Do not let stand, test immediately.
	Refrigerated: Unacceptable
	Frozen: Unacceptable
Timing Considerations	Process immediately.
Unacceptable Specimens & Actions to Take	Dry swab. Call and request another sample
Compromising Physical Characteristics	N/A
Other Considerations	N/A

4. REAGENTS

N/A

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

N/A

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

Microscope

7.3 Supplies

Sterile transfer pipette
Paper towels
Glass slide
Coverslip
Gloves

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Action
1.	Check order and verify patient name in the LIS matches name on specimen.
2.	Place a drop or two of the specimen on a slide using a plastic transfer pipette.
3.	Gently place a coverslip over the surface of the material on the slide.
4.	Examine with low (10x objective) and high dry (40x objective) power objectives.
5.	Enter results in computer utilizing Microbiology Result Entry.

9. CALCULATIONS

N/A

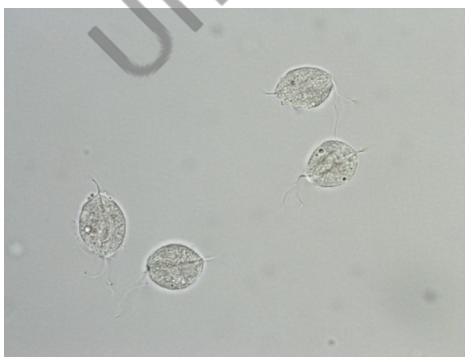
10. REPORTING RESULTS AND REPEAT CRITERIA

** See procedure AHC.M28- Resulting Microbiology Direct Exams on how to result.

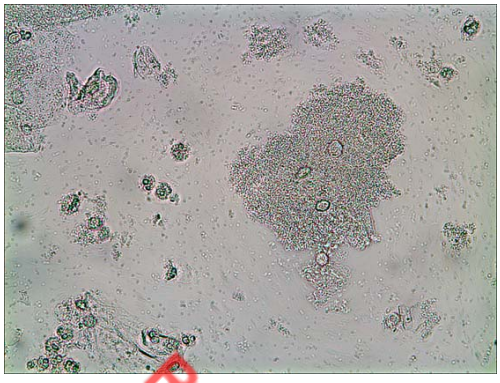
10.1 Interpretation of Reporting of Results

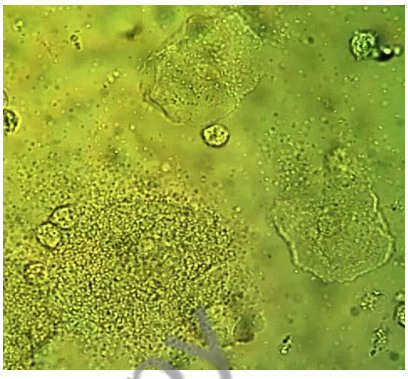
Report the presence or absence of *Trichomonas*, yeast and clue cells.

- In wet mounts the trophozoites of *T. vaginalis* move with a rapid, jerky motion and possess an undulating membrane that extends half the length of the organism. They are pear-shaped, 7-23 µm long and 5-15 µm wide. There are 3-5 anterior flagellae and 1 posterior flagella.

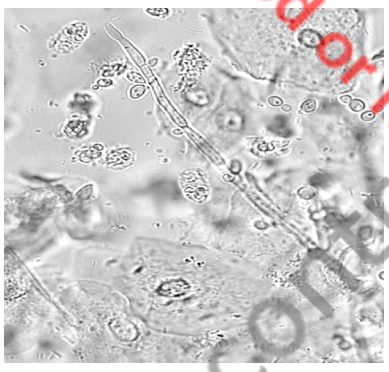


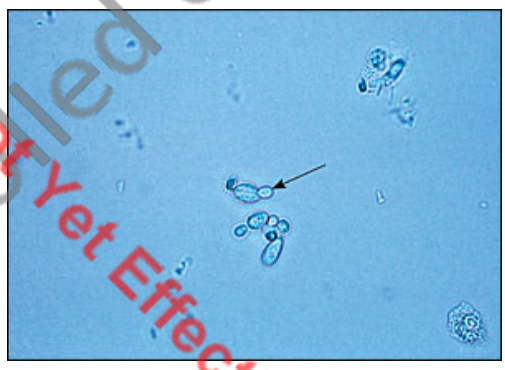
- Clue cells are squamous epithelial cells from the vagina with many small rod shaped bacteria adherent to their surfaces. **NOTE**: Bacteria must extend beyond the cytoplasmic margins to be considered a clue cell.





- Yeast are small, ovoid cells which often have small buds or pseudohyphae attached.





DO NOT REPORT SPERMATOZOA.

11. EXPECTED VALUES

11.1 Reference Ranges

No Trichomonas, yeast, or clue cells seen

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Trichomonas vaginalis is a parasite that is distinguished by its rapid movement and flagella. Motile *Trichomonas* trophozoites may be identified in a vaginal sample by its characteristic structure.

Bacterial vaginosis is the most common type of vaginal infection and can sometimes be detected by the presence of "clue cells". Clue cells are epithelial cells entirely covered with bacteria giving them a "furlike" appearance. If the organisms sticking to the edges or on top of the cell, without extending past the cytoplasmic margins, a diagnosis of clue cells cannot be made. Note: Certain anaerobic, non-pathogenic, species tend to adhere to the epithelial surface.

Yeast vaginitis is primarily caused by *Candida albicans*, although other *Candida* species are becoming increasingly important as disease agents. *Candida albicans*, in low numbers, is considered part of the normal vaginal flora, but may proliferate to cause an infection.

13. PROCEDURE NOTES

- **FDA Status:** LDT without message
- **Validated Test Modifications:** None

1. Examine wet preparation immediately as motility disappears rather rapidly (within 35-40 minutes) making it impossible to detect the parasite in wet preparations.

2. *Trichomonas vaginalis* can also be observed on Gram stain of the specific discharge or sedimented urine. Here the *Trichomonas* appear larger than the polymorphonucleated white cells, but smaller than epithelial cells. *Trichomonas* cytoplasm is typically foamy in appearance; the parasite stains slightly pink, demonstrating its typical pear to oval or any shape. The elliptical nucleus is clearly visible as it stains darker than the cytoplasm of the organism. Flagella are sometimes visible but not always.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

N/A

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

AHC.M28 Resulting Microbiology Direct Exams, Microbiology procedure

17. REFERENCES

- Baron, Ellen Jo, Sydney Finegold, Bailey and Scott's Diagnostic Microbiology, C.V. Mosby Co., St. Louis, .2002.
- P.C. Beaver, R.C. Jung, E.W. Cupp. *Clinical Parasitology*, 9th Edition, p. 49-51, Lee and Febiger Publishers, Philadelphia, PA, 1984.
- Henry, J.B, Clinical Diagnosis and Management by Laboratory methods, 19th ed., W. B. Saunders Company, Philadelphia, 1996
- Quest Diagnostics Incorporated procedure *Wet Mount (Vaginal) for Clue Cells, Yeast and Trichomonas vaginalis*, QDRRL820v2

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP M010.002		
000	10/12/09	8.1	LIS update to GUI system	A. Sears	R. Master
000	10/12/09	16	Added procedure for resulting	L. Barrett	R. Master
001	10/4/2011	3.2	Deleted sources other than vaginal	R. Master	R. Master
002	5/16/2012	10.1	Deleted hyphae, deleted redundant report comments	R. Master	R. Master
003	7/6/16	Header	Added other sites	L. Barrett	R. Master
003	7/6/16	3.2	Specify saline concentration	R. Master	R. Master
003	7/6/16	11.1	Corrected reference range	R. Master	R. Master
003	7/6/16	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L. Barrett	R. Master
4	7/10/18	10.1	Added photos	L. Barrett	R. Master
4	7/10/18	15	Updated to new standard wording	L. Barrett	R. Master
4	7/10/18	17	Added RRL SOP	L. Barrett	R. Master
5	7/6/20	Header	Changed WAH to WOMC	L. Barrett	R. Master
6	7/7/22	Header	Changed site to All Laboratories	D Collier	R. Master
6	7/7/22	Footer	Changes prefix to AHC	D Collier	R. Master

7	7/12/22	Section 10	Added SOP reference on how to result Added more info defining Clue Cells	M Sabonis D Collier	R. Master
7	7/12/22	Section 10 and 16	Added related document and changed SOP prefix to AHC	M Sabonis D Collier	R. Master

19. ADDENDA

None

AHC.M28 Resulting Microbiology Direct Exams

Copy of version 4.0 (approved, not yet effective)

Last Approval or Periodic Review Completed	8/2/2022
Next Periodic Review Needed On or Before	8/2/2024
Effective Date	8/23/2022

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Organization Adventist HealthCare

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	8/2/2022	4.0	Nicolas Cacciabeve	
Approval	Laboratory Operations Director	8/2/2022	4.0	Robert SanLuis Robert SanLuis	
Approval	Lab Director	11/9/2020	3.0	Nicolas Cacciabeve	
Approval	Micro Director approval	11/6/2020	3.0	Ronald Master	
Approval	QA approval	11/6/2020	3.0	Leslie Barrett	
Periodic review	Designated Reviewer	12/26/2018	2.0	Robert SanLuis Robert SanLuis	
Approval Captured outside MediaLab	Lab Director	3/24/2017	2.0	Nicolas Cacciabeve	Recorded on 11/21/2018 by Leslie Barrett when document added to MediaLab
Periodic review Captured outside MediaLab	Designated Reviewer	3/24/2017	2.0	Nicolas Cacciabeve	Recorded on 11/21/2018 by Leslie Barrett when document added to MediaLab

Approvals and periodic reviews that occurred before this document was added to the MediaLab Document Control system may not be listed.

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
4.0	Approved, Not Yet Effective	Major revision	8/1/2022	8/23/2022	Indefinite
3.0	Approved and Current	Major revision	11/6/2020	11/9/2020	8/23/2022
2.0	Retired	First version in Document Control	11/21/2018	4/10/2017	11/9/2020

Non-Technical SOP

Title	**Resulting Microbiology Direct Exams**	
Prepared by	Marie Sabonis	Date: 10/8/2009
Owner	Marie Sabonis, Ronald Master	Date: 10/8/2009

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Issue Date:	Local Effective Date:	

TABLE OF CONTENTS

1. PURPOSE

Describe the procedure for resulting Microbiology direct exams.

2. SCOPE

Applies to the resulting of gram stains (non-blood cultures), wet preps and malaria smears. To assist in resulting, certain orderables are tied to resulting keyboard.
Example:

Orderable	Keyboard
Gram Stain(GS)	MDE
CSF Culture and gram stain (XCSFC)	MDE
Fluid culture and gram stain(XFLC)	MDE
Wound culture and gram stain(XWDCG)	MDE
Malaria(MAL)	DES2
Wet Prep(WET)	WPDE

3. RESPONSIBILITY

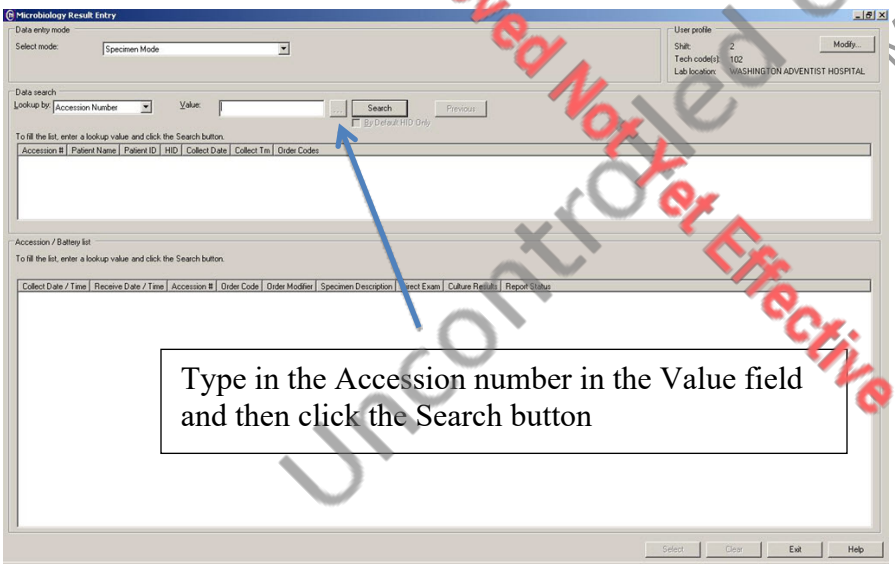
It is the responsibility of all personnel assigned to Microbiology and Lead technologists to read, understand and perform this procedure.

4. DEFINITIONS

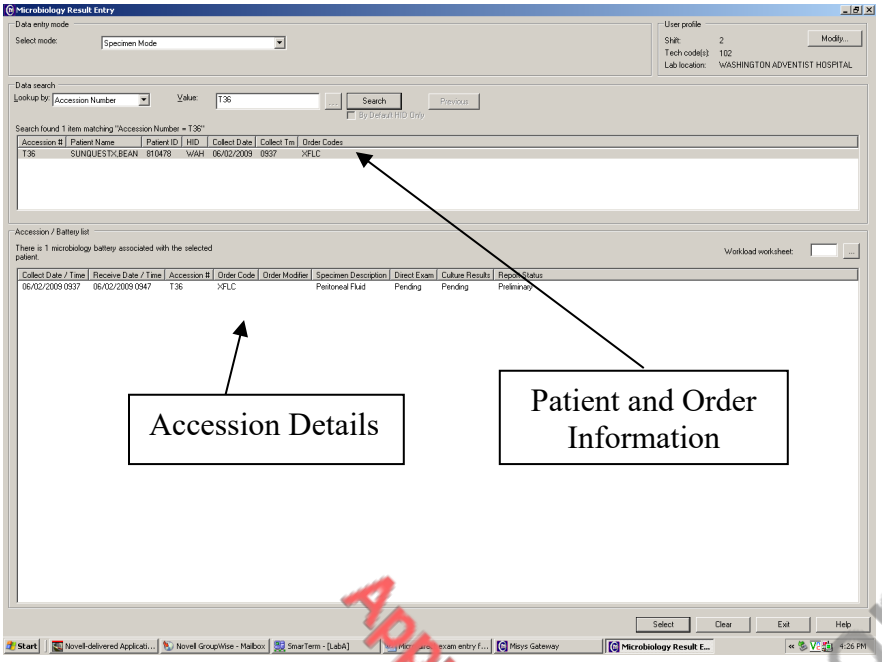
None

5. PROCEDURE

1. Access the GUI Microbiology Result Entry via the Misys Gateway application.
2. The first screen is divided into three sections
   - Data Entry Mode
   - Data Search
   - Accession / Battery search
3. In the Data Entry Mode section, keep the default of specimen mode
4. In the Data Search section, key in the accession number you want to result in the "Value" field and click the search button or press enter. See below

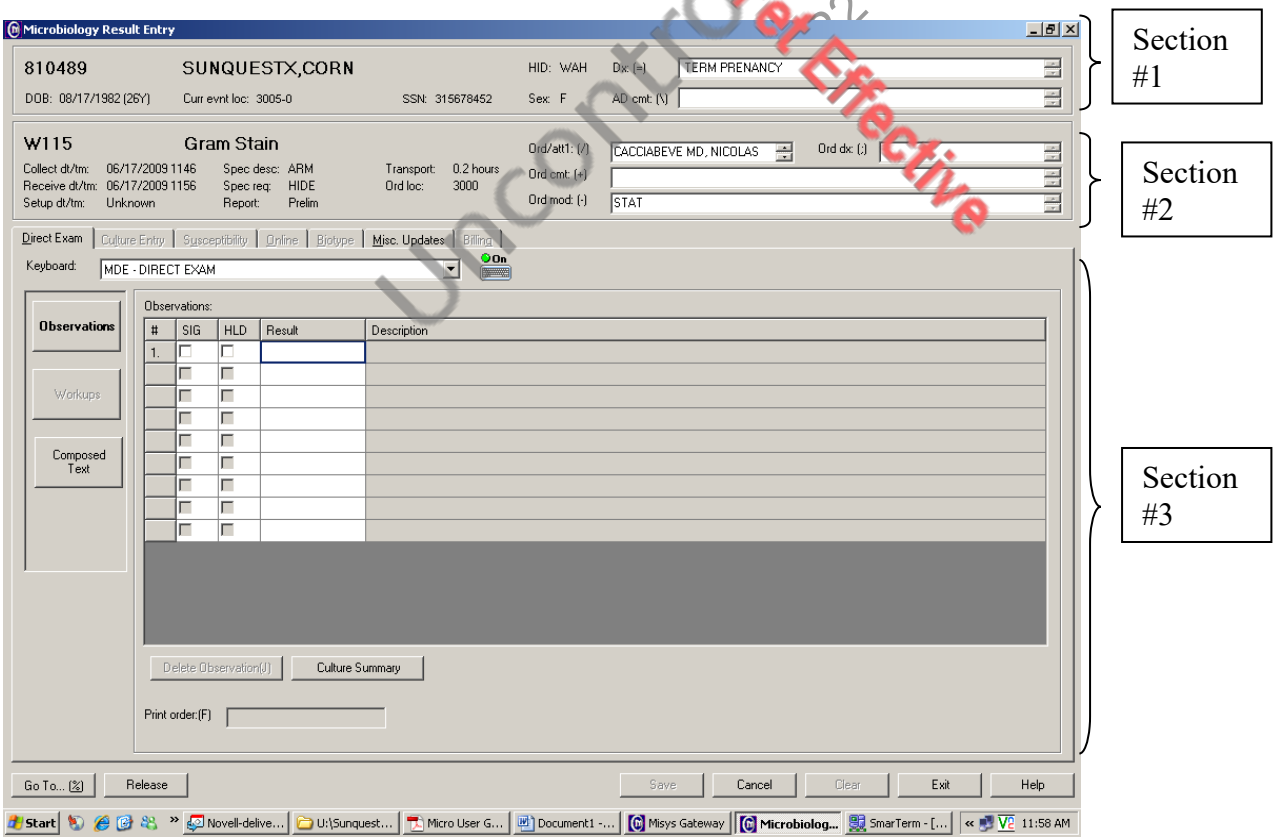


5. The following will now display on the screen. The center section will provide the patient information and the Order code. The lower section will display information in regards to the accession.



6. If this is the accession that you want to result, click on the "**Select**" button at the bottom of the screen or press "**ALT**" + "**S**". You will now enter into the actual result screen for the direct exam.

7. Microbiology Result Screen display:



Micro Result Entry Screen is divided into three components.

a. Section 1: Patient Demographics
Located on top of screen, includes patient current location (Curr Evnt Loc)

b. Section 2: Accession number information, includes
1) Collect/Receive date/time
2) Source (Spec Desc, Spec Req)
3) Report: This provides the status of the report, i.e., Prelim or Final
4) Patients location at time of order is noted here (Ord Loc)
5) Attending physician
6) Order Mod: Displays the priority code for the order.

c. Section 3: Resulting
This is the result section. Note, that on the top of this section there are tabs **bolded,** denoting that they are associated with the order.
1) **Misc Update** - if you click on this tab, the specimen description and special request display. If you need to change the specimen description you can do it from here.
2) **Direct Exam -** This tab is where you would result a gram stain, wet prep or malaria smear.

8. Resulting
a. To result, your cursor must be in the result field.

b. Make sure your keyboard is on (keyboard icon will say "on" with a green dot). This allows you to use the preprogrammed keyboard codes. If the keyboard is not displayed on the screen you can press "F8" to pull it up. You can move the keyboard on the screen to any location that you wish by moving your mouse to the banner section of the keyboard ("Direct Exam Result/Modifier keys"). Hold left mouse button down and "drag and drop" the keyboard where you want it.

c. The drop down menu labeled 'keyboard' will default to the correct keyboard code for the test to be resulted.
The MDE keyboard is used to result Gram stains.
The DES2 keyboard is used to result Malaria.
The WPDE keyboard is used to result wet preps.

See addenda for detailed keyboard descriptions.
To access a keyboard other than the default, open the drop down menu and select from the list.

Gram Stain Keyboard (MDE):

Malaria Keyboard (DES2):

Wet Prep(WPDE):

d. Enter your first observation either by using the actual keyboard **or** point and click the keyboard displayed on the screen.
Example: If you want to enter "few WBCs", select the number **2** key and then select the **W** key. Note that both entries display on the same observation line but are linked together.
Enter one observation (**quantity + organism or cell type**) per observation line (always enter the quantity first.)
The translation of what you entered displays on the right hand side of the screen.

e. To get to the next Observation, use the tab key to tab over to the next result field or use your mouse or click on the next line.

f. To enter English Text codes or Free Text:
1) English Text - Enter ";" (semicolon) in an observation line. This turns off the keyboard codes (keyboard icon says OFF) and brings up the ellipsis box ... . Click on the ellipsis to bring up the Select Text Code screen and enter the correct English text code.
2) Free Text - Enter ";" (semicolon) to turn off the keyboard codes and then a second ";" to allow Free Texting. Enter result.
3) When you are finished, click on another result field to turn keyboard entry back on and continue result entry.

g. After entering all your observations, you must save your results as a preliminary or final result.

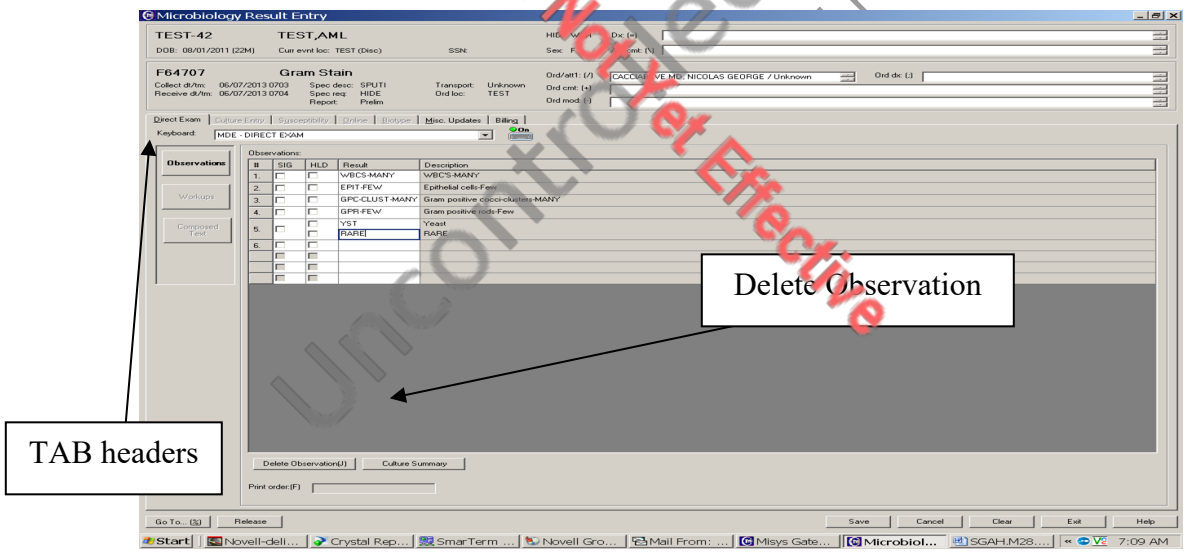
1) To save the direct exam as a preliminary:

Click on **save** at the bottom of the screen or press "**ALT**"+"**S**". Do NOT select the final key (/) from your keyboard or the keyboard displaying on the screen.

2) To finalize the direct exam:
Make sure the cursor is in a blank result field, then select the final key "/" from your keyboard or the keyboard displaying on the screen.
   i. A message will display on the screen stating the direct exam has been finalized and the status will change on the second section of the screen.
   ii. Select '**Save the results**' or press "**ALT**"+"**S**" Before exiting.
   iii. If you attempt to exit before saving, a message will prompt: "**You have unsaved data. Do you want to discard it?**" If you select "Yes" then everything that you entered will be lost.

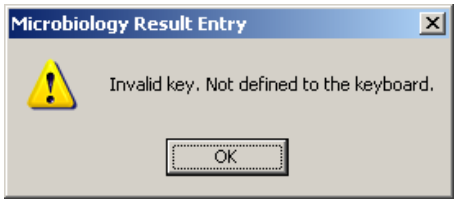
Notes:

1. If you have entered a direct exam observation and you want to change it
   a. Press the DELETE key while in the result field that contains the observation results that you want to delete. Example, if you entered in the wrong quantity or organism.
   b. If you want to delete everything on an observation line then click on "Delete Observation [J]" or "ALT"+"J"



2. TAB headers. Note that there is an underscore associated with the Tab headers for Direct Exam and Misc Updates. The underscore lets you know that you can use the ALT key plus the underscored letter to navigate over to that section/area.

3. If you try to enter an observation and the following message displays:



Check the CAPs Lock on your keyboard. It may be off.

4. Once you have verified a result you return to the screen where you can select the next patient or accession number to result. A "Previous" button now appears next to the "Select" button. This will take you back to the last patient or accession number you worked on depending on whether you are under Patient ID or Acc Number. This will reopen the patient in the Misc Updates tab, to return to the Direct Exam screen click the Direct Exam tab (see above).

5. If a Direct Exam is finalized in error you can return to the Result entry screen and enter **"/"** to unfinalize the results. When corrected results are entered, press **"/"** again to re-finalize.

6. RELATED DOCUMENTS

None

7. REFERENCES

Sunquest Microbiology Admin Manual

8. REVISION HISTORY

Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SOP LIS047.001		
000	6/6/13	Section 5: In step 8.d - change order for reporting an observation by replacing (organism or cell type + quantity) with (quantity + organism or cell type) Notes 1.b - update Result screen print.	M Sabonis	R Master
001	3/20/17	Header: add other sites Section 5: update keyboard screen shots in step 8.c Section 9: update Appendix A & B Footer: version # leading zero's dropped due to new EDCS in use as of 10/7/13	M Sabonis	R Master
2	11/6/20	Header: changed WAH to WOMC	L Barrett	R Master
3	8/1/22	Section 4 Definition removed- n/a Section 7 Reference-removed version # of user manual Section 8c Added updated screen shot of Wet Prep keyboard Added appendix C- Keyboard translation for Wet preps	M Sabonis	R SanLuis
3	8/1/22	Header: Changed site to All Laboratories Footer: Changed prefix to AHC	D Collier	R SanLuis

9. ADDENDA AND APPENDICES

A: Gram Stain and Wet Prep Keyboard Codes
B: Malaria Keyboard Codes
C: Wet Prep Codes

Appendix A Gram Stain and Wet Prep Keyboard Codes

KEY	TRANSLATION	CODE
/	<finalize>	(fnl)
—	<do not report>	HIDE
;	<free text> <English text codes>	(other)
F	Branching gram positive rods	BRPR
J	chains	CHAIN
Q	Clue cells	CLUE
K	clusters	CLUST
?	Corrected report, previously reported as	PREVR
E	Epithelial cells	EPIT
2	Few	FEW
D	Gram negative diplococci	GNDC
C	Gram negative cocci	GNC
M	Gram negative coccobacilli	GNCB
I	Gram negative rods	GNR
H	Gram positive cocci	GPC
G	Gram positive coccobacilli	GPCB
U	Gram positive rods	GNR
S	Gram positive rods resembling diptheroids	GPRD
V	Gram variable coccobacilli	GVCB
B	Gram variable rod	GVR
7	Hyphal elements seen	HYPH
4	Many	MANY
3	Moderate	MOD
N	Negative	NEG
5	No cell or organisms seen	NOCO
O	No organisms seen	NOS
6	No Trichomonas, yeast or clue cells seen	NTY
L	pairs	PAR
P	Positive	POSIT
1	Rare	RARE
R	RBC's	RBCP
Z	The sputum specimen submitted contains 25 or more squamous epithelial cells per low power field and is unacceptable for culture due to oropharyngeal contamination. Please resubmit another specimen if clinically indicated.	SPUU
T	Trichomonas	TRIC
W	WBC's	WBC
Y	Yeast	YST
8	Yeast with pseudohyphae	YPSU

Appendix B Malaria Keyboard Codes

KEY	MALARIA KEYBOARD TRANSLATION	CODE
M	Parasitemia	INF2
/	<FINAL>	(fnl)
N	negative	NEG
B	No parasites seen. One set of blood films cannot exclude the diagnosis of malaria.	NMAL1
A	Plasmodium falciparum	PLAF
D	Plasmodium malariae	PLAM
R	Plasmodium species not P. falciparum	PLSNF
E	Plasmodium vivax/ovale	PLAVO
F	Plasmodium ovale	PLAO
G	Plasmodium species	PLA
I	Unable to identify species due to low parasitemia.	UNABLE
S	Plasmodium vivax	PLAV
L	Blood Flagellate	BFLAG
P	Positive	POSIT
T	Microfilaria	MFIL
K	Plasmodium species, unable to identify species	PSUID
H	Presumptive positive, confirmation and identification to follow	PMAL1
?	Corrected report, previously reported as	PREVR
J	Thin smear presumptive negative, thick smear and final report to follow	NMLP1

Appendix C **Wet Prep Keyboard Codes**

KEY	TRANSLATION	CODE
/	<finalize>	(fnl)
—	<do not report>	HIDE
;	<free text> <English text codes>	(other)
6	No Trichomonas, yeast or clue cells seen	NTY
E	Trichomonas present	TRP
D	Yeast present	YSTP
C	Clue cells present	CSP
U	Trichomonas not seen	TRKN
K	Yeast not seen	YSTN
M	Clue cells not seen	CNS
?	Corrected report, previously reported as	PREVR