Ministry of Health

Document Title	MRSA screening SOP
Document Type	Procedure
Directorate/Institution	The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC)
Targeted Group	Medical Laboratories
Document Author	Dr Nawal Al-Kindi
Designation	Consultant medical microbiologist
Document Reviewer	1. Dr. Mahmoud Al Subhi 2. Ms. Zainab Al Hadhrami
Designation	1. Consultant medical microbiologist **2.** Technologist specialist A
Release Date	May 2023
Review Frequency	Three years

Validated by		**Approved by**	
Name	Dr. Muna Habib	**Name**	Dr.Badryah Al Rashidi
Designation	Director Department Development & Conterol (DGQAC)	**Designation**	Director General of Primary Health Care
Signature		**Signature**	
Date	May 2023	**Date**	June 2023

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Acknowledgment

The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH) would like to thank and appreciate the great effort of the Microbiology documents development team. Participated and contributed personnel are:

Member name	**Institution**	**Designation**
Dr.Mahmoud Al Subhi	Rustaq Hospital	Team Leader Consultant medical microbiologist
Ms. Zainab Al Hadhrami	Directorate General of Specialized Medical Care	Team Coordinator Senior technologist specialist A
Ms. Saleh Al Shukairi	Ibra Hospital	Senior technologist specialist A
Dr. Hanaa Al Auraimi	Royal Police of Oman Hospital	Consultant medical microbiologist
Dr. Nawal AL Kindi	Khoula Hospital	Consultant medical microbiologist
Dr. Al Warith Al Kharusi	Nizwa Hospital	Consultant medical microbiologist
Dr. Abdulrahman Al Mahrouqi	Ibri Hospital	Specialist microbiologist pathologist
Dr. Nada Al Tamimi	Al Massara Hospital	Consultant medical microbiologist
Dr. Wafaa Al Tamtami	Armed Forces Hospital	Specialist microbiologist pathologist

Contributors:

- Dr. Muna Al Mahrouqi, medical officer

Acronyms:

BA	Blood agar
CA	Chocolate agar
MAC	macConkey
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
QC	Quality Control
MDRO	Multidrug Resistant Organism
MRSA	Methicilin Resistant Staph. Aureus
SOP	Standard operating procedure
TAT	Turnaround time
WHO	World Health Organization

1. **Purpose**

   This document describes the procedure for processing specimens for MRSA screening.

2. **Scope**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions**

   3.1 MRSA: *Staphylococcus aureus* strains that are resistant to penicillin and other beta lactams.

   3.2 MRSA screening: detecting colonization with MRSA strains

   3.3 MRSA selective media: media that are selectively made to detect MRSA strains.

4. **Procedure**

   4.1.Clinical background:

   MRSA strains are a continuing problem in healthcare settings, with transmissions and outbreaks occurring in healthcare setting and in the community. Screening for MRSA provides means of identifying patients and staff who may be at risk of infection and/or involved in transmission of the organism. In order to achieve the most effective use of hospital resources and to minimize morbidity, it is mandatory to have a policy of planned screening to guide control measures to protect patients from MRSA colonization and infection.

   4.2. Principle:

   - Ideally, a screening method should allow the growth of all MRSA, inhibit or differentiate other organisms, and allow direct identification tests to be performed on colonies.
   - Direct plating on selective medium like chromogenic agars has the advantage that results may be available within 24hr, but most studies indicate that direct plating is less sensitive than broth enrichment followed by plating on solid media.
   - According to the risk assessment, MRSA screening swabs are important specimens that should be requested and processed prior intra-operation stage to rationalize the pre-operation antibiotic prophylaxis when needed.
   - Infection prevention and control team should be notified immediately for a positive preliminary results of MRSA screening specimens.

4.3.Pre – analytical stage:

4.3.1. Specimen collection, transport and storage

4.3.1.1 MRSA screening specimens are usually combination of three swabs from different body sites including nose, throat, axilla, groin or perineum.

4.3.1.2 Swabs should be collected in appropriate transport medium with charcoal.

4.3.1.3 Dry swabs should not be used unless specimens can be transported immediately to the laboratory.

4.3.1.4 Swabs should be transported to the laboratory immediately through porters or pneumatic tube in sealed plastic bags.

4.3.1.5 Ensure that expired transport media is not used specimen is clearly labelled with the patient's name, number, date and time of collection, and type of sample.

4.3.1.6 Compliance with transport and storage regulations is essential.

4.3.1.7 If processing of swabs is delayed, refrigeration is preferable to storage at ambient temperatures. Delays of over 48 hours are undesirable.

4.3.1.8 Specimens for molecular methods should follow manufacturer's instructions. Specimens should be transported and processed as soon as possible.

4.3.1.9 All specimens are stored for one week (+/-) according to lab storage capacity.

4.3.2. Material:

Reagents	**Consumables/Supplies**	**Equipment**
Gram stain reagents Blood agar Chromogenic agars Mueller Hinton agar Cefoxitin discs staphylococcal latex kit coagulase test Gram Staining kit 0.5 McFarland standard 0.85% sterile saline	- 10 ul disposable loops - Cleaned glass slides - Sterile Pasteur pipettes - Bunsen burner - Staining rack	- Class 2 Biosafety cabinet - Hot plate - Light Microscope - Automated ID machine - Aerobic Incubator (35 - 37°C)

4.3.3. Safety precaution:

4.3.3.1 All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages.

4.3.3.2 Laboratory procedures must be conducted in a microbiological safety cabinet Class 2.

4.3.3.3 Fix smeared material by placing the slide on an electric hotplate (65-75°C), inside the safety cabinet, until dry.

4.3.3.4 Hands should be thoroughly washed with soap and water before and after handling all specimens.

4.3.3.5 Disinfect all work surfaces with 70% alcohol or a freshly prepared 10% bleach solution prior to testing and after processing.

4.3.3.6 All samples and reagents should be properly discarded according to the current standards for disposal of hazardous waste.

4.3.3.7 Samples and culture plates should be autoclaved before finally discarding.

4.3.4. Quality control:

4.3.4.1 All reagents, kits, stains and media MUST pass the quality check before use. All tests are run with appropriate controls as applicable.

4.3.4.2 Results of all tests are invalid if the QC test results are not as expected.

4.3.4.3 Ensure that reagents, stains and media are not used beyond the expiry date.

4.3.4.4 Check all reagents and stains before use to ensure that they are free from contamination, debris or deposits.

4.3.4.5 All media used should be examined visually just before use to ensure that there is no contamination or deterioration appearing as lysis, discoloration, drying, shrinkage, or cracking of the media.

4.3.4.6 Use the ATCC control organisms for the QC check.

4.3.4.7 The ability to recover MRSA using chrome agar procedure could be assessed for new batches as follows:

1. Subculture daily a selective chrome agar with 10^{3} CFU of MRSA - *SAUR (ATCC 43300)* using sterile culturing loop (positive control) and with methicillin sensitive staphylococcus aureus strain (negative control).
2. Incubate the plate in 37 C for 18-24 hrs of incubation in a box.
3. Chrome agar plates should not be exposed to light for long time.
4. Inspect the chrome agar for the dark pink colony pigmentation.
5. White or colorless colonies considered negative and has to be conformed using Staphurex agglutination test.

4.4.Analytical stage:

4.4.1. Swabbing

- Use of correct swabbing technique has been shown to improve bacterial recovery rate. Pooled swabs may be used.
- Enrichment broth containing 7% NaCl may inhibit the growth of some isolates of MRSA if present in small numbers. For this reason, 2.5% NaCl is recommended as it has been shown to work well when sub culturing to chromogenic agar.

4.4.1.1 Direct method using chromogenic agar:

1. Inoculate media and streak for individual colonies.

2. Incubate aerobically at 35 - 37°C for 24 hours.
3. Check for pink colonies on chromogenic agar or according to manufactured recommendations.
4. Gram stain typical colonies to confirm Gram-positive cocci.
5. Perform either a slide or tube coagulase test (or commercial latex test).
6. If coagulase or latex test positive, report as a presumptive MRSA.

4.4.1.2 Direct culture method using Blood Agar:

1. Inoculate 5% sheep blood agar plate and streak for individual colonies using a sterile loop.
2. Incubate aerobically or in 5% CO at 35 - 37°C for 18 -24 hours.
3. Check for white, cream, yellow or golden colonies.
4. Perform Gram stain to confirm Gram-positive cocci.
5. Perform modified oxidase test to rule out Micrococcus sp. (Micrococcus sp. is oxidase positive; Staphylococcus sp. is oxidase negative).
6. Perform catalase test using 3% hydrogen peroxide.
7. If catalase positive; perform tube coagulase or staphylococcal latex test.

4.4.1.3 Indirect culture method (pooling method):

1. Take the 3 swabs and the (3) ml sterile saline broth to the safety cabinet.
2. Remove the glass tube cap from the container and inoculate the swabs plate (all of the three swabs one by one) by tapping and squeezing the swabs in the tube to ensure best extraction of the MRSA, and replace the cap.
3. Vortex for 20 seconds.
4. Streak the chrome selective MRSA medium in the safety cabinet using 10 ul disposable loop.
5. Incubate the plates at **37°C** aerobically for 18 to 48** hours (don't expose the plates to direct light during incubation.
6. Read daily for 2 days.
7. Record the 24 hrs and 48 hrs results in MRSA work list.

8. Test the suspected colony for staphurex latex agglutination and Send the positive staphurex isolate for susceptibility and identification.

4.4.2. Culture

4.4.2.1.Follow the culture conditions as per table:

Clinical details/ conditions	**Specimens**	**Media**	**Incubation**			**Cultures read**	**Target organisms**
			Temp ºC	**Atmos**	**Time**		
Direct culture	MRSA screening specimens	Chromogenic selective MRSA medium	35-37	Aerobic	18 - 48hr*	Daily	MRSA
OR							
Indirect culture method	MRSA screening specimens	A broth containing 2.5% NaCl then subculture to Chromogenic Or selective MRSA medium	30	Aerobic	18-24hr	N/A	
			35-37	Aerobic	18-48hr	Daily	MRSA
***** Molecular methods may be considered if a rapid result is required							

4.4.3. Identification

4.4.3.1 Identification should be performed by automated systems or conventional biomedical tests. It is essential that the organism is identified to species level to enable correct interpretation of test results.

4.4.3.2 Minimum level of identification in the laboratory: S. aureus species level, cefoxitin resistant. Identification can follow local laboratory.

4.4.3.3 Molecular methods: Different commercial kits use different combinations of MRSA genetic specific targets. Assessment of available methods indicated good performance and results in 2-3hr even using in house methods.

4.4.3.4 Other methods used in MRSA screening: Other methods giving more rapid results may be considered, such as the latex agglutination-based method that detects the PBP2a protein which is commercially available.

4.4.4. Antimicrobial susceptibility testing

4.4.4.1 The susceptibility of MRSA strains from screening samples should follow the CLSI guidelines.

4.4.4.2 Susceptibility should be reported if required.

4.4.4.3 The susceptibility can be done either in automated ID machines when available or manual using disc diffusion methods as follows:

1. Prepare the inoculum with the suspected colonies by emulsifying in saline and standardising to a 0.5 McFarland turbidity standard.
2. Inoculate Mueller Hinton plate for confluent growth using a swab which has been dipped into the inoculum prepared above.
3. Place a cefoxitin 30µg/ml disk on to the plate and incubate at 35 - 37o C for 18 - 24hrs.
4. Read plates and measure zone of inhibition.
5. Report susceptibilities as clinically indicated.
6. If confirmed, perform vancomycin susceptibility using MIC or E-test. Note: Vancomycin is the treatment of choice for MRSA. Other beta-lactam agents, such as penicillins, beta lactam/beta lactamase inhibitor combinations, cephalosporins and carbapenems may appear active in vitro but are not effective clinically. Results for these drugs using manual disc tests, should be reported as resistant or should not be reported.

4.5.Post – analytical stage:

4.5.1. Reporting:

4.5.1.1 when the identification is done by automated machine:

Negatives : “MRSA not detected”

Positives : “MRSA detected ” , along with the susceptibility report if required and a comment mentioning the importance of notifying and adhering to infection prevention and control precautions.

4.5.1.2 When the identification is done manually using the disc diffusion methods :

Cefoxitin zone size ≥22mm = MRSA not detected

Cefoxitin zone size ≤21mm = MRSA detected along with the susceptibility report if required and a comment mentioning the importance of notifying and adhering to infection prevention and control precautions.

5. Responsibilities

5.1. Responsible staff:

5.1.1 To ensure the results are verified by microbiologist or pathologist.

5.1.2 To ensure adherence to critical result communication procedure.

5.1.3 To facilitate the alternative communication channels once needed.

5.2.Quality manager /officer

5.2.1 To follow up the implementation of the procedure.

5.2.2 To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3.All lab staff:

5.3.1 To adhere to the procedure.

5.3.2 To document record and release results as recommended.

5.3.3 To report test failures or incident.

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
B_29i7.pdf retrieved from: https://assets.publishing.sevice.gov.uk/government/uploads/system/uploads/attachment_data/file/887595/B_29i7.pdf		cited 2022 Sep 25	
MRSA_Isolation_Identification.pdf [Internet]. [Available from: https://www.cmedlabsfoundation.com/wp content/uploads/2020/07/microbiology/tech_methods/MRSA_Isolation_Identification.pdf		Cited 2022 Sep 25	