INSTRUCTIONS FOR USE

RPMI AGAR

Ready-to-use plates

RPMI Agar: *Candida krusei* and voriconazole gradient-strip

1 - INTENDED USE

In vitro diagnostic device. Culture medium for quantitative determination of susceptibility to antifungal agents by gradient-based strips.

2 - COMPOSITION - TYPICAL FORMULA *

RPMI 1640	10.4 g
MOPS **	34.5 g
Glucose	20.0 g
Agar	15.0 g
Purified water	1000 mL

* The formula may be adjusted and/or supplemented to meet the required performances criteria.
** 3-(N-morpholino) propanesulfonic acid

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The reference tests for antifungal susceptibility testing are the broth microdilution assays devised by the Clinical and Laboratory Standards Institute (CLSI)[1] and by the European Committee on Antibiotic Susceptibility Testing (EUCAST) [2,3,4] These reference methods are time-consuming and poorly suited for the routine clinical laboratory setting.[5] To overcome these limitations some methods based on gradient strip testing have been developed and are commercially available for the laboratory. Such tests are based on the diffusion of a stable concentration gradient of an antimicrobial agent from a plastic or paper strip onto an agar medium. The medium devised for the detection of MICs with gradient-based strips is RPMI 1640 supplemented with agar, glucose and buffered with MOPS. This medium derives from the formulation recommended for the broth microdilution assays.[1]
RPMI 1640 was developed by Moore, Gerner, and Franklin[6] in 1967 at Roswell Park Memorial Institute, from where it derives its name. It contains vitamins, amino acids, salts, a pH indicator and it is widely used for cell cultures. When supplemented with MOPS, glucose and agar, RPMI 1640 demonstrated accurate MICs with antifungal agents on gradient-based strips, comparable with the results obtained with CLSI reference method.[4, 7-11]

4 - PHYSICAL CHARACTERISTICS

Medium appearance	pink, limpid
Final pH at 20-25 °C	7.0 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
RPMI Agar	Ready-to-use plates	54RPMI90	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box
RPMI Agar	Ready-to-use plates	54RPMI15	5 plates ø 150 mm primary packaging: cellophane sachet secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, gradient-based strips with antifungal agents.

7 - SPECIMENS

RPMI Agar must be used with pure culture of fungal strains isolated from clinical specimens. RPMI Agar is not intended for microbial isolation directly from clinical specimens.

8- TEST PROCEDURE

Allow plates and antifungal gradient strips to equilibrate to room temperature. The surface of the agar should be dry before use.
Guidelines for inoculums preparation:
Inoculum for yeast: suspension in saline to 0.5 McFarland for *Candida* spp. and 1 McFarland for *C.neoformans*.
Inoculum for moulds: suspension of both conidia and hyphae (mature growth 5-7days) in saline with Tween to 0.5 McFarland for Aspergillus spp. e 1 McFarland for *Fusarium* and *Rhizopus* spp.
Dip a sterile cotton swab into the suspension and remove excess fluid by pressing and turning the swab against the inside of the tube.
With the cotton swab inoculate evenly the entire surface of the plate, taking care to check that t that there are no gaps between streaks. Dip the swab again and inoculate a second time. Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator.
As soon as the inoculum has been absorbed and the agar surface is dry, apply the gradient-strips. Make sure that the strips are in complete contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from strips is very rapid. The strips should be placed on the agar plate in a manner which does not result in overlapping zones of inhibition.

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Guidelines for incubation:
Yeast: 35°C in air for 24-48 hours for *Candida* spp. and 48-72 hours for *C.neoformans*.
Aspergillus spp.: 35°C for 18-24 hours; *Fusarium* spp.: 35°C/24-48 hours, followed by room temperature for 24-48 hours; *Rhizopus* spp.: 35°C for 18-24 hours
For other species, extend the incubation time as needed, inspect plates daily for the formation of readable inhibition ellipse.
For the details of inoculation and incubation procedures consult the gradient-strips manufacturer's package insert.

9 - Reading and interpretation

After incubation, read plates from the front with the lid removed and with reflected light.
A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light and the test must be repeated.
The growth should be evenly distributed over the agar surface to achieve a uniform inhibition ellipse.
Check that inhibition zones for quality control strain are within acceptable range.
Determination of the MIC is at the point at which the lower part of the bacterial growth ellipse intersects with the corresponding number on the test strip. For specific reading and interpretation instructions consult the gradient-strips manufacturer's package insert.

10 - User quality control

All manufactured lots of RPMI Agar plates are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. For QC organisms testing, details are described in the strips manufacturer's package insert. At a minimum, at least one QC strain should be tested to ensure proper product functionality. Strain and the gradient-strips useful for the quality control: *C.parapsilosis* ATCC 22019 / amphotericin B, voriconazole. Refer to interpretation guidelines in IFU provided by manufacturer of antifungal gradient-strips.

ATCC is a trademark of American Type Culture Collection.

11- Performances characteristics

Prior to release for sale a representative sample of all lots of ready-to-use plates of RPMI Agar is tested by Antimicrobial Sensitivity Testing. AST is performed according to gradient-strips manufacture's IFU with the following strains and antifungal gradient strips: *C.krusei* ATCC 6258, *C.parapsilosis* ATCC 22019, *C.albicans* ATCC 90028/ amphotericin B and voriconazole gradient-strips.
After incubation at 35-37°C in air for 48 hours, MIC is read where the inhibition ellipse intersects the MIC scale zones and the measures are recorded and evaluated to be within the quality control ranges reported by strips manufacturer's package insert.

12 - Limitations of the method

- Incorrect inoculum concentration, improper storage of antimicrobial strips, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.
- The inoculation, incubation and reading methods here described are to be considered as guidelines; strict adherence to the protocol suggested by gradient-strips manufacturer is required to ensure reliable results.
- Reading and interpretation require expertise and close adherence to the gradient-strips manufacturer instructions; problems can arise when inexperienced readers incorrectly interpret faint background growth of small colonies within the zones as resistance.[12]
- Agreement of gradient-strips testing and reference MICs medium may be species, drug and medium dependent.[13,14]
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - Precautions and warnings

- RPMI Agar is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- RPMI Agar is not classified as dangerous according to current European legislation.
- All laboratory specimens should be considered infectious.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - Storage conditions and shelf life

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

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15 - References

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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/01
Revision 2	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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