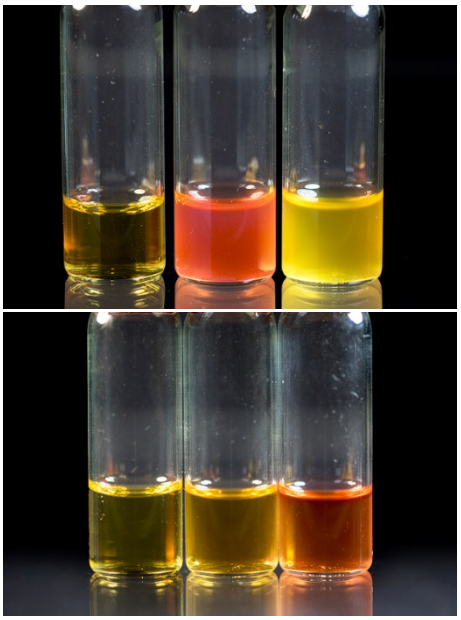
INSTRUCTIONS FOR USE

MRVP MEDIUM

Dehydrated culture medium



MRVP Medium:
on top: MR test (uninoculated tube, *E.coli* +, *E.aerogens* -)
at left: Voges-Proskauer test (uninoculated tube, *E.coli* -, *E.aerogenes* +)

1 - INTENDED USE

In vitro diagnostic. For the differentiation of bacteria by means of the methyl red and Voges Proskauer reactions.

2 - COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	7
Glucose	5
Phosphate buffer	5

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Voges and Proskauer[1] in 1898 and Clark and Lubs[2] in 1915 were the first bacteriologists to observe that the gas produced during fermentation was a mix of CO_2 and H_2 and the red colour reaction produced in appropriate culture medium after treatment with potassium hydroxide. Clark and Lubs optimized the culture medium proposing a formulation with 0.5% peptone, 0.5% glucose and, 0.5% K_2HPO_4, that is the base of the current MRVP medium.
Both methyl red and Voges-Proskauer tests are commonly used in conjunction with the indole and citrate tests, to form a group of tests known as IMViC which aid in the differentiation of *Enterobacteriaceae*, but are now also used to characterize other groups of bacteria including *Actinobacteria*.[3] The medium is recommended by FDA BAM for MR and VP tests in the identification procedures of *Salmonella*.[4]
Escherichia coli and other members of the low-ratio organisms described by Clark and Lubs, those which produce a low ratio of CO_2 to H_2 from the fermentation of glucose, ferment glucose by the mixed acid pathway resulting in a large quantity of acids produced (lactic, succinic, acetic, formic acids) exceeding the buffer system of the medium and lowering the pH to values below 4.4.[3] *Enterobacter aerogenes* and other members of the high-ratio of CO_2 to H_2 organisms, ferment sugars via the butanediol pathway, producing only 1 mol of acid per mol of glucose. This pathway results in a lower degree of acidification of the culture medium.[3] The pH indicator methyl red has been found to be suitable to measure the concentration of hydrogen ions between pH 4.4 (red) and 6.0 (yellow).[5]
The Voges-Proskauer test determines the ability of bacteria to ferment glucose via the butanediol pathway. The pyruvate that is formed by glycolysis in the Embden-Meyerhof pathway, is transformed into α-acetolactate, then into acetoin and finally into butanediol. In ~~the~~ presence of oxygen and KOH, the intermediate acetoin is oxidized to diacetyl, a reaction which is catalyzed by α-naphthol. Diacetyl reacts with the guanidine groups of the arginine contained in the peptones, giving rise to a pinkish-red-coloured product. α-naphthol and creatine are catalysts and intensifiers of the chromatic reaction.
For lactose fermenting enteric bacteria there is almost always a negative correlation between MR test and VP test: *Escherichia coli* is MR positive and VP negative, *Enterobacter aerogenes* is MR negative and VP positive.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 17 g in 1000 mL of cold purified water. Heat slightly to dissolve, with frequent agitation, dispense 5 mL in screwcap tubes and sterilize by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	beige, limpid
Final pH at 20-25 °C	6,9 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
MRVP Medium	Dehydrated culture medium	4017352	500 g (29.4L) CND: W0104010101; EDMA 14.01.01.01;RDM: 1873890/R

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave and water-bath, sterile loops, incubator and laboratory equipment as required, ancillary culture media and reagents for the MR and VP tests and for complete identification of the culture.

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8 - SPECIMENS

MRVP Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

9 - TEST PROCEDURE

Transfer a light inoculum from an isolated colony and re-suspend it in the tube. Note that the use of a heavy inoculum may result in aberrant results.
Incubate in aerobic atmosphere for a minimum of 48 hours and up to 5 days at 35 ± 2°C.
For a rapid VP test, with heavy inoculation, it is possible to incubate at 35°C in a water bath for 4 hours.[6]

10 - READING AND INTERPRETATION

One medium is used for two test procedures, MR and VP; the MR test is performed after 48 hours or longer of incubation, the VP test usually after 24 and 48 hours. Therefore, it is necessary to test aliquots for the VP test. In case it is necessary to prolong incubation for MR test, it is recommended that an aliquot also be used for the MR test.[6]

1- Methyl Red test (MR)

Aseptically, remove a 2.5 mL aliquot and add 5 drops of Methyl Red Reagent. Interpret colour result immediately.
Positive test: red colour at the surface of the medium. Negative test: yellow colour at the surface of the medium.
In case of delayed reaction, indicated by the development of an orange colour, continue the incubation to 5 days and repeat the test.

2- Voges-Proskauer Test (VP)

Aseptically, remove a 2.5 mL aliquot for the Barrit test[7], or 1 mL for the O'Meara test[8], or 0.2 mL for the rapid Barry and Feeney test[9].
2A - Barrit test: to 2.5 mL of MRVP Medium add 0.6 mL of Barrit Reagent A and 0.2 mL of Barrit Reagent B; shake gently after each addition to aerate the culture. Read after 10-15 minutes.
Positive test: pinkish red colour at the surface of the medium.
Negative test: yellow colour at the surface of the medium; a coppery colour is a sign of negative test.
2B - O'Meara test: to 1 mL of MRVP medium incubated at 35°C for 4 hours in a water-batch, add 1 mL of O'Meara reagent. Shake the tube gently 30 sec to 1 min to expose the medium to oxygen in order to oxidize the acetoin.
Positive test: pinkish red colour at the surface of the medium.
Negative test: yellow colour at the surface of the medium; a coppery colour is a sign of negative test.
2C - Barry and Feeney rapid test: to 0.2 mL of MRVP Medium inoculated with a single colony from selective medium, incubated at 35°C for 4-6 hours, add 2 drops of Barry and Feeney Reagent, 2-3 drops of Barrit Reagent A and 2-3 drops of Barrit Reagent B. Shake the tubes after each addition of reagent. Read after 15 minutes.
Positive test: cherry red colour within 15 minutes.
Negative test: yellow colour at the surface of the medium.

3-Reagents

Methyl Red Reagent: completely dissolve 0.1 g of methyl red in 300 mL of ethanol (95%). Add 200 mL of purified water to make 500 mL.
Barrit Reagent A: 5 g of α-naphthol in 100 mL of absolute ethyl alcohol.
Barrit Reagent B: 40% KOH in purified water;
O'Meara reagent: 40% KOH + 0.3% creatine in purified water.
Barry and Feeney Reagent: 0.3% creatine in purified water

In the table below, adapted from Edwards and Ewing[10] and MacFaddin[6], the MR/VP results for *Enterobacteriaceae*

Microorganism	MR	VP
Escherichia coli	+	-
Shigella	+	-
Edwardsiella tarda	+	-
Salmonella spp.	+	-
Salmonella arizonae	+	-
Citrobacter spp	+	-
Klebsiella ozoaenae	+	-
K.rhinoscleromatis	+	-
Morganella morganii	+	-
Proteus vulgaris	+	-
Providencia spp	+	-
Klebsiella pneumoniae	-	+
Enterobacter aerogenes	-	+
Enterobacter cloacae	-	+
Serratia marcescens	V	+
Hafnia alvei (35°C)	V+	V
Yersinia enterocolitica	+	- (35°C)
Proteus mirabilis	+	V-
Klebsiella oxytoca	V+	V-

V+: variable, usually positive; V-: variable, usually negative; V: variable

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E.coli ATCC 25922	33-37°C / 48H / A	MR + / VP -
K.pneumoniae ATCC 27736	33-37°C / 48H / A	MR - / VP +

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

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12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated MRVP Medium is tested for performances characteristics comparing the results with a previously approved Reference Batch.
Pure colonies cultivated on Tryptic Soy Agar of 7 *Enterobacteriaceae* strains are lightly inoculated into the tubes: *E.coli* ATCC 25922, *C.freundii* ATCC 8090, *E.cloacae* ATCC 13047, *E.aerogenes* ATCC 13048, *K.pneumoniae* ATCC 27736, *S.*Thyphimurium ATCC 14028, *S.flexneri* ATCC 12022. After incubation at 33-37°C for 48 hours aerobically, the appropriate reagents are added to each test tube and the color changes are observed. All strains show reactivity according to the specifications for both batches tested.

13 - LIMITATIONS OF THE METHOD

- The results of the MR and VP tests must be used together with other biochemical tests to differentiate the genera and species within the *Enterobacteriaceae*.
- For the MR test, in order to obtain reproducible results, each laboratory should standardize the inoculum density, the total volume of the broth and the size of the test tubes. Sometimes an orange reaction occurs when using too large volume of broth.[6]
- The reaction of methyl red cannot be accelerated by increasing the glucose concentration of the medium.[6]
- Read the MR test not earlier than 48 hours of incubation. If the MR test is performed too early the results are often equivocal or falsely positive.[6] Incubate MR-negative tests for more than 48 hours and test again.
- Read the VP test after 48 hours of incubation. Longer incubations can produce acidic conditions in the broth that will interfere with reading the results. Only in the case of a negative test continue the incubation.[6]
- Do not automatically consider a VP positive strain as MR negative or vice versa; although it is true in most cases, there are certain organisms such as *Hafnia alvei* and *Proteus mirabilis*, which can be positive in both tests, even if the VP reaction is often delayed.[6]
- It is important to follow the order indicated for the addition of Barrit's A and B reagents: first α-naphthol, then KOH. A reversal of order of reagents addition may give weak positive or false negative reactions.
- Do not exceed the volume of 0.2 mL of 40% KOH, since the excess of the reagent can mask a weak VP positive reaction by exhibiting a copper-like colour due to its reaction with α-naphthol.[6]
- The maximum colour for the VP positive test occurs 1 hour after adding the reagents. By exceeding the time of 1 hour, the negative strains may show a copper-liker colour, leading to false positive results. According to Vaughn[11], these false positives are more frequent with incubation at 30°C rather than at 35°C.
- After each addition of the α-naphthol and KOH reagents, the test tubes must be gently shaken to expose the broth to atmospheric oxygen to promote the oxidation of acetoin, if present, to diacetyl.[6]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis 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.

14 - PRECAUTIONS AND WARNINGS

- This product 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.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

16 - 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	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 3	Updated layout and content	2020/06

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

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