

MacConkey HiVeg™ Media

MacConkey HiVeg Media are differential media recommended for the selection and recovery of *Enterobacteriaceae* and related enteric gram-negative bacilli. The following media are available.

1. (MV008) MacConkey HiVeg™ Agar without CV, with 0.003% NR and 1.5% Agar
2. (MV008A) MacConkey HiVeg™ Agar without CV, with 0.0075% NR and 1.2% Agar
3. (MV008B) MacConkey HiVeg™ Agar without CV with 0.003% NR and 1.2% Agar
4. (MV081) MacConkey HiVeg™ Agar with CV, NaCl, 0.003% NR and 1.5% Agar
5. (MV081A) MacConkey HiVeg™ Agar with CV, NaCl, 0.005% NR and 1.5% Agar
6. (MV081B) MacConkey HiVeg™ Agar with CV, NaCl, 0.003% NR and 1.35% Agar
7. (MV082) MacConkey HiVeg™ Agar without CV and NaCl, with 0.004% NR and 2.0% Agar
8. (MV082A) MacConkey HiVeg™ Agar without CV and NaCl, with 0.0075% NR and 1.2% Agar
9. (MV051) MacConkey HiVeg™ Agar, Modified
10. (MV061) MacConkey HiVeg™ Agar with Bromo Thymol Blue
11. (MV298) MacConkey Sorbitol HiVeg™ Agar
12. (MV007) MacConkey HiVeg™ Broth with Neutral Red
13. (MV083) MacConkey HiVeg™ Broth Purple with BCP

They suppress a number of gram-positive bacteria including *Staphylococci*.

MV082 : MacConkey HiVeg Agar is the modification of MacConkey Agar prepared as per Medical Microbiology by Cruickshank et al (2). It is a differential medium which restricts the swarming of most *Proteus* species thereby permitting greater ease in the detection and isolation of enteric organisms. It is specially useful for culturing urine specimens which may contain large number of *Proteus* species as well as potentially pathogenic gram-positive organisms. *Enterococci* produce compact tiny reddish colonies either on or beneath the surface.

MV082A : It is a modification of MV082 containing Synthetic detergent and reduced amount of agar.

MV051 : This agar medium is used in membrane filtration procedure for the detection and isolation of *Klebsiella* species from water and other sources.

MV061 : This medium has almost the same composition as MV008 except that the indicator is bromothymol blue instead of neutral red.

MV298 : It contains sorbitol instead of lactose and is recommended for detection of enteropathogenic *Escherichia coli* strains (3).

MV007 : This medium is a modification of MacConkey Broth with Neutral Red (M007) which is considered as a standard medium for the primary isolation as well as presumptive identification of coliform-aerogenes group of organisms in food and water. The medium contains neutral red as an indicator.

MV083 : This broth has similar use as MV007 except that it contain bromo cresol purple as an indicator instead of neutral red (4, 5). This liquid medium is meant for presumptive identification of coliforms from a variety of specimens such as water, milk and food.

Intended Use :

MV008, MV008B and MV008A : These are modifications of MacConkey Agar prepared without crystal violet. These are slightly less selective than the formula of MacConkey HiVeg Agar (MV081).

MV081, MV081A and MV081B : These agar media contain crystal violet and are very selective compared to MV008.

Formulae of different MacConkey Media

Ingredients (Grams/Litre)	MV008	MV008A	MV008B	MV081	MV081A	MV081B	MV082
HiVeg peptone	17	23	17	1.5	20	1.5	23
HiVeg peptone No. 3	3	-	3	-	-	-	-
HiVeg hydrolysate	-	-	-	1.5	-	1.5	-
HiVeg peptone No. 2	-	-	-	17	-	17	-
Lactose	10	10	10	10	10	10	10
Sorbitol	-	-	-	-	-	-	-
Synthetic detergent	1.5	2	1.5	1.5	1.5	1.5	-
Synthetic detergent No. V	-	-	-	-	-	-	2
Sodium chloride	5	5	5	5	5	5	-
Crystal violet	-	-	-	0.001	0.001	0.001	-
Neutral red	0.03	0.075	0.03	0.03	0.05	0.03	0.04
Agar	15	12	12	15	15	13.5	20
pH (at 25°C) ± 0.2	7.1	7.4	7.1	7.1	7.2	7.1	7.4
Grams per litre	51.53	52.07	48.53	51.53	51.55	50.03	55.04

Continued...

MacConkey HiVeg™ Media

Ingredients (Grams/Litre)	MV082A	MV298	MV051	MV061	MV007	MV083
HiVeg peptone	23	17	17	17	23	23
HiVeg peptone No. 3	-	3	3	3	-	-
Lactose	10	-	-	10	10	10
Inositol	-	-	10	-	-	-
Sorbitol	-	10	-	-	-	-
Synthetic detergent	2	1.5	1.5	1.5	2	-
Synthetic detergent No. V	-	-	-	-	-	2
Sodium chloride	-	5	5	5	5	5
Crystal violet	-	0.001	0.001	-	-	-
Neutral red	0.075	0.03	0.03	-	0.075	-
Bromo cresol purple	-	-	-	-	-	0.01
Bromo thymol blue	-	-	-	0.03	-	-
Agar	12	13.5	13.5	15	-	-
pH (at 25°C) ± 0.2	7.4	7.1	7.1	7.1	7.4	7.4
Grams per litre	47.07	50.03	50.03	51.53	40.07	40.01

Directions :

A) For MacConkey HiVeg Agars : Suspend the appropriate quantity of medium as specified on the label, in 1000 ml distilled water. Heat to boiling with gentle swirling to dissolve the agar completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45 - 50°C and pour into sterile petriplates. The surface of the medium should be dry when inoculated. For MacConkey HiVeg Agar, Modified (MV051), autoclaving is not recommended. After boiling, cool the medium to 50°C, and add 50 mg Carbenicillin per litre and mix well before pouring in sterile petri plates.

B) For MacConkey HiVeg Broths : Suspend the appropriate amount of medium as specified on the label, in 1000 ml distilled water. Heat to dissolve the medium completely. Distribute into tubes with inverted Durham's tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes before inoculation. Incubate the inoculated media aerobically at 35 - 37°C. After 18 - 24 hours, examine agar plates and the broth tubes for growth, acid and gas production.

Principle and Interpretation :

These media are prepared by using vegetable peptone in place of animal based peptones which makes the medium free of BSE/TSE risks. MacConkey HiVeg Agar is the modification of MacConkey Agar which is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (7, 8). Subsequently MacConkey HiVeg Agar and Broth like the conventional medium have been recommended for use in microbiological examination of foodstuffs (9) and for direct plating / inoculation of water samples for coliform counts (10, 14). Medium contains protein, synthetic detergents, sodium chloride and two dyes. The selective action of this

medium is attributed to crystal violet and synthetic detergents, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink coloured colonies. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature. Sorbitol MacConkey HiVeg Agar is the modification of conventional Sorbitol MacConkey Agar which is based on the formulation described by Rappaport and Henig (3). In this medium lactose has been replaced by sorbitol as it is a recommended medium for isolation of enteropathogenic *Escherichia coli* 0157:H7. *Escherichia coli* 0157:H7 does not ferment sorbitol and hence produces colourless colonies. This organism has been recognized as a cause of haemorrhagic colitis (12). Sorbitol MacConkey HiVeg Agar however should not be used solely to detect pathogenic *Escherichia coli* 0157:H7 strains as some non-toxic strains will also not ferment sorbitol. It is necessary to select suspected colonies for further identification. MacConkey HiVeg Agar, Modified (MV051) is the modification of medium which is prepared according to the formulation recommended in Standard Methods (10). *Klebsiella* species are associated with coliforms in water supply distribution systems and are often present as a major component in industrial wastes of paper mill, textile and other industries. The normal coliform population in human and other warm blooded animal faeces may contain 30 to 40% *Klebsiella* strains. Out of this population 4% are of bacterial pneumonia cases and 18% of urinary tract. Infections are caused by pathogenic

Continued...

MacConkey HiVeg™ Media

strains of *Klebsiella*. In this medium inositol is incorporated in place of lactose while added Carbenicillin makes the medium selective for *Klebsiella species*. Further, this method reduces the necessity for biochemical testing of pure strains, however, preliminary verification of differentiated colonies is recommended.

Quality Control :

**MacConkey HiVeg™ Agar without CV,
with 0.003% NR and 1.5% Agar**

MV008

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity

Light red coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.15% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

**MacConkey HiVeg™ Agar without CV,
with 0.0075% NR and 1.2% Agar**

MV008A

Appearance of Powder :

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity

Light red coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.2% w/v aqueous solution is pH 7.4 ± 0.2 at 25°C.

**MacConkey HiVeg™ Agar without CV
with 0.003% NR and 1.2% Agar**

MV008B

Appearance of Powder:

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity

Light red coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 4.85% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

**MacConkey HiVeg™ Agar with CV, NaCl,
0.003% NR and 1.5% Agar**

MV081

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity

Red with purplish tinge, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.15% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

**MacConkey HiVeg™ Agar with CV, NaCl,
0.005% NR and 1.5% Agar**

MV081A

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity

Red with purplish tinge, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.15% w/v aqueous solution is pH 7.2 ± 0.2 at 25°C.

**MacConkey HiVeg™ Agar with CV, NaCl,
0.003% NR and 1.35% Agar**

MV081B

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity

Red with purplish tinge, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.0% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

**MacConkey HiVeg™ Agar without CV and NaCl,
with 0.004% NR and 2.0% Agar**

MV082

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity

Red coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.5% w/v aqueous solution is pH 7.4 ± 0.2 at 25°C.

**MacConkey HiVeg™ Agar without CV and NaCl,
with 0.0075% NR and 1.2% Agar**

MV082A

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Continued...

MacConkey HiVeg™ Media

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity

Red coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 4.7% w/v aqueous solution is pH 7.4 ± 0.2 at 25°C.

MacConkey Sorbitol HiVeg™ Agar

MV298

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity

Purplish red coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.0% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

MacConkey HiVeg™ Agar, Modified

MV051

Appearance of Powder

Pinkish beige coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity

Purplish red coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.0% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

MacConkey HiVeg™ Agar with Bromo Thymol Blue

MV061

Appearance of Powder

Yellow coloured may have slightly greenish tinge, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity

Green coloured, clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.15% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

MacConkey HiVeg™ Broth with Neutral Red

MV007

Appearance of Powder

Yellow coloured may have slightly greenish tinge, homogeneous, free flowing powder.

Colour and Clarity

Red coloured, clear solution without any precipitate.

Reaction

Reaction of 4.0% w/v aqueous solution is pH 7.4 ± 0.2 at 25°C.

MacConkey HiVeg™ Broth Purple with BCP

MV083

Appearance of Powder

Yellow coloured may have slightly greenish tinge, homogeneous, free flowing powder.

Colour and Clarity

Purple coloured, clear solution without any precipitate.

Reaction

Reaction of 4.0% w/v aqueous solution is pH 7.4 ± 0.2 at 25°C.

Cultural Response :

MV008 and MV008B

Cultural characteristics observed after an incubation at 35-37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Enterobacter aerogenes</i> (13048)	10 ² - 10 ³	luxuriant	>50%	pink
<i>Escherichia coli</i> (25922)	10 ² - 10 ³	luxuriant	>50%	pink - red
<i>Proteus vulgaris</i> (13315)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Enteritidis</i> (13076)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Typhi</i> (6539)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Paratyphi A</i>	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Paratyphi B</i>	10 ² - 10 ³	luxuriant	>50%	colourless
<i>Shigella flexneri</i> (12022)	10 ² - 10 ³	fair-good	>30%	colourless
<i>Enterococcus faecalis</i> (29212)	10 ² - 10 ³	none-poor	<10%	pale pink - red
<i>Staphylococcus aureus</i> (25923)	10 ² - 10 ³	inhibited	0%	—

MV081, MV081A and MV081B

Cultural characteristics observed after an incubation at 35-37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Enterobacter aerogenes</i> (13048)	10 ² - 10 ³	luxuriant	>50%	pink to red
<i>Escherichia coli</i> (25922)	10 ² - 10 ³	luxuriant	>50%	pink to red
<i>Proteus vulgaris</i> (13315)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Enteritidis</i> (13076)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Typhi</i> (6539)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Paratyphi A</i>	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Paratyphi B</i>	10 ² - 10 ³	luxuriant	>50%	colourless
<i>Shigella flexneri</i> (12022)	10 ² - 10 ³	fair-good	>30%	colourless
<i>Enterococcus faecalis</i> (29212)	10 ² - 10 ³	inhibited	0%	—
<i>Staphylococcus aureus</i> (25923)	10 ² - 10 ³	inhibited	0%	—

MV082, MV082A and MV008A

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Enterobacter aerogenes</i> (13048)	10 ² - 10 ³	luxuriant	>50%	pink - red
<i>Escherichia coli</i> (25922)	10 ² - 10 ³	luxuriant	>50%	pink - red
<i>Proteus vulgaris</i> (13315)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Enteritidis</i> (13076)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Typhi</i> (6539)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Paratyphi A</i>	10 ² - 10 ³	luxuriant	>50%	colourless
<i>S. serotype Paratyphi B</i>	10 ² - 10 ³	luxuriant	>50%	colourless
<i>Shigella flexneri</i> (12022)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>Enterococcus faecalis</i> (29212)	10 ² - 10 ³	good	>30%	pale pink - red
<i>Staphylococcus aureus</i> (25923)	10 ² - 10 ³	good	>30%	pale pink - red

Continued...

MacConkey HiVeg™ Media

MV051

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Klebsiella pneumoniae</i> (13883)	10 ² - 10 ³	luxuriant	>50%	pink
<i>Escherichia coli</i> (25922)	10 ² - 10 ³	inhibited	0%	—
<i>Enterobacter aerogenes</i> (13048)	10 ² - 10 ³	inhibited	0%	—
<i>S. serotype Typhi</i> (6539)	10 ² - 10 ³	inhibited	0%	—
<i>Serratia marcescens</i> (8100)	10 ² - 10 ³	inhibited	0%	—

MV061

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Enterobacter aerogenes</i> (13048)	10 ² - 10 ³	luxuriant	>50%	yellow
<i>Escherichia coli</i> (25922)	10 ² - 10 ³	luxuriant	>50%	yellow
<i>Proteus vulgaris</i> (13315)	10 ² - 10 ³	luxuriant	>50%	colourless – light blue
<i>S. serotype Typhi</i> (6539)	10 ² - 10 ³	luxuriant	>50%	colourless – light blue
<i>Shigella flexneri</i> (12022)	10 ² - 10 ³	luxuriant	>50%	colourless – light blue
<i>Enterococcus faecalis</i> (29212)	10 ² - 10 ³	inhibited	0%	—
<i>Staphylococcus aureus</i> (25923)	10 ² - 10 ³	inhibited	0%	—

MV298

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Escherichia coli serotype</i> 011 and 055, (pathogenic)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>Escherichia coli</i> (25922)	10 ² - 10 ³	luxuriant	>50%	pale pink–red
<i>S. serotype Typhi</i> (6539)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>Shigella flexneri</i> (12022)	10 ² - 10 ³	luxuriant	>50%	colourless
<i>Escherichia coli</i> 0157:H7	10 ² - 10 ³	luxuriant	>50%	colourless

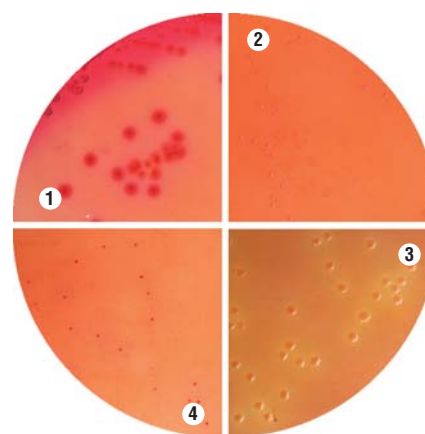
MV007 and MV083

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Acid	Gas
<i>Enterobacter aerogenes</i> (13048)	10 ² - 10 ³	luxuriant	+	+
<i>Escherichia coli</i> (25922)	10 ² - 10 ³	luxuriant	+	+
<i>S. serotype Choleraesuis</i> (12011)	10 ² - 10 ³	fair–good	—	—
<i>Staphylococcus aureus</i> (25923)	10 ² - 10 ³	inhibited	—	—

References :

- Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), Standard Methods for the Examination of Water and Wastewater, 1985, 16th ed., A.P.H.A., Washington, D.C.
- Rappaport F. and Henigh E., 1952, J. Clin. Path., 5:361.
- International Organization for Standardization (ISO), 1990, Draft ISO/DIS 9308-2.
- Harrigan W.F. and McCance M.E. (Eds.), 1976, Laboratory Methods in Food and Dairy Microbiology, Academic Press, London.
- Holt, Harris and Teague, 1916, J. Infect. Dis., 18:596.
- MacConkey, 1900, The Lancet, ii:20.
- MacConkey, 1905, J. Hyg., 5:333.
- Speck M. (Ed.), 1985, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.
- Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 1992, Standard Methods for the Examination of Water and Wastewater, 18th ed., APHA, Washington, D.C.
- Marshall R. (Ed.), 1992, Standard Methods For the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
- Karmali M.A., Petric M., Lim C., et al, 1985, J. Infect. Dis., 151:775.
- Lior H. and Borczyk A., 1987, Lancet, i:333.
- MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.



MV082 MacConkey HiVeg Agar without CV and NaCl, with 0.004% NR and 2.0% Agar

- | | |
|---------------------------------|-------------------------------------|
| 1. <i>Escherichia coli</i> | 3. <i>Salmonella serotype Typhi</i> |
| 2. <i>Staphylococcus aureus</i> | 4. <i>Enterococcus faecalis</i> |