V-C-M Medium

INTENDED USE

Quest V-C-M Medium is intended for the collection and transport of clinical specimens containing viruses, chlamydiae, mycoplasmas or ureaplasmas from the collection site to the testing laboratory. This system can be processed using standard clinical laboratory operating procedures for viral, chlamydial, mycoplasmal and ureaplasmal culture.

SUMMARY AND EXPLANATION

One of the routine procedures in the diagnosis of infections caused by viruses, chlamydiae, mycoplasmas or ureaplasmas involves the collection and safe transportation of biological samples. This can be accomplished using the Quest V-C-M Medium. This system includes a universal transporting medium that is room temperature stable, which can sustain viability (and infectivity) of a plurality of organisms that include clinically important viruses, chlamydiae, mycoplasmas and ureaplasmas during transit to the testing laboratory. The formulation of Quest V-C-M Medium includes protein for stabilization, antibiotics to minimize bacterial and fungal contamination, and a buffer to maintain a neutral pH.

Quest V-C-M Medium is provided with labeled capture-cap vials designed for transport of the clinical sample. The capture-cap is designed to secure the shaft of the swab sample to the cap, eliminating the use of forceps to remove the swab in the laboratory.

PRINCIPLES OF THE PROCEDURE

Quest V-C-M Medium consists of modified Hank's balanced salt solution supplemented with bovine serum albumin, cysteine, gelatin, sucrose and glutamic acid. The pH is buffered with HEPES buffer. Phenol red is used to indicate pH. Vancomycin, amphotericin B and colistin are incorporated in the medium to inhibit growth of competing bacteria and yeast. The medium is isotonic and non-toxic to mammalian host cells. The presence of sucrose acts as a cryoprotectant which aids in the preservation of viruses and chlamydiae if specimens are frozen (–70°C) for prolonged storage.

REAGENTS

V-C-M Medium Components

Hank's Balanced Salts
Bovine Serum Albumin
L-Cysteine
Gelatin
Sucrose
L-Glutamic Acid
HEPES Buffer
Vancomycin
Amphotericin B
Colistin
Phenol Red
pH 7.3 ± 0.2 @ 25°C

Warnings and Precautions

For *in vitro* Diagnostic Use.

- Inserting more than one swab in the capture-cap vial may interfere with proper cap closure.
- Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified personnel.
- Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[1-4] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.
- Sterilize all biohazard waste including specimens, containers and media after their use.
- Directions should be read and followed carefully.
- Do not re-pack.
- Not suitable to collect and transport microorganisms other than viruses, chlamydiae, mycoplasmas and ureaplasmas.
- Not suitable for any other application than intended use.
- The use of this product in association with a rapid diagnostic kit or with diagnostic instrumentation should be previously validated by the user.
- Do not ingest the medium.
- Do not use the V-C-M Medium for premoistening or prewetting the applicator swab prior to collecting the sample or for rinsing or irrigating the sampling sites.
- Do not use for more than one patient.
- Avoid skin contact with medium.

Storage: This product is ready for use and no further preparation is necessary. The product should be transported and stored in its original container at 2–25°C until used. Do not overheat. Do not incubate or freeze prior to use. Improper storage will result in a loss of efficacy. Do not use after expiration date, which is clearly printed on the specimen transport vial label.

Product Deterioration: Quest V-C-M Medium should not be used if (1) there is evidence of damage or contamination to the product, (2) there is evidence of leakage, (3) the color of the medium has changed from light orange-red, (4) the expiration date has passed, or (5) there are other signs of deterioration.

SPECIMEN COLLECTION AND PREPARATION

Specimens for viral, chlamydial, mycoplasmal or ureaplasmal investigation should be collected and handled following published manuals and guidelines.[5-11] To maintain optimum viability, transport the specimen to the laboratory as soon as possible. Best recovery is obtained when specimens are refrigerated at 2–8°C or kept on wet ice following collection and while in transit. If there will be a long delay before processing, specimens should be frozen at –70°C or colder and transported on dry ice. Storage at –20°C is less satisfactory than storage at 4°C or –70°C and can result in the loss of infectivity.[12,13] Specific requirements for the shipment and handling of specimens should be in full compliance with state and federal regulations.[11,14,15] Shipping of specimens within medical institutions should comply with internal guidelines of the institution. All specimens should be processed as soon as they are received in the laboratory.

PROCEDURES

Materials Provided: Quest V-C-M Medium includes a capture-cap vial containing 3 mL of transport medium plus three glass beads.

Materials Required But Not Provided: Specimen collection swab, appropriate materials for isolating, differentiating and culturing viruses, chlamydiae, mycoplasmas and ureaplasmas. These materials include tissue culture cell lines, tissue culture medium, incubation systems and reading equipment. Refer to appropriate references for recommended protocols for isolation and identification of viral, chlamydial, mycoplasmal and ureaplasmal agents.[5-8,10]

Test Procedure

Proper specimen collection from the patient is extremely critical for successful isolation and identification of infectious organisms. For specific guidance regarding specimen collection procedures, consult published reference manuals.[5-11] Specimens should be collected as soon as possible after the clinical onset of disease. Highest viral titers are present during the acute illness.

For V-C-M Medium Vials

1. Aseptically remove cap from vial.
2. Aseptically place sample into the vial with medium.
3. Replace cap on vial and close tightly.
4. Label with appropriate patient information.
5. Send to the laboratory for immediate analysis.

Quality Control

All lots of the V-C-M Medium are tested for microbial contamination, toxicity to host cells and the ability to maintain viability of desired agents. Procedures for quality control of V-C-M Medium and viral culture media are described in a number of publications by the American Society for Microbiology[6,8,10] and by CLSI (formerly NCCLS).[16,17] If aberrant quality control results are noted, patient results should not be reported.

RESULTS

Results obtained will largely depend on proper and adequate specimen collection, as well as timely transport and processing in the laboratory.

LIMITATIONS OF THE PROCEDURE

1. Condition, timing and volume of specimen collected for culture are significant variables in obtaining reliable culture results. Follow recommended guidelines for specimen collection.[5-11]
2. Repeated freezing and thawing of specimens may reduce the recovery of viable organisms.
3. V-C-M Medium is intended for use as a collection and transport medium for viral, chlamydial, mycoplasmal and ureaplasmal agents only. The medium can serve as a cryoprotectant for clinical viruses, including cytomegalovirus and varicella-zoster virus.
4. Calcium alginate swabs are toxic for many enveloped viruses and may interfere with fluorescent antibody tests, so they should not be used for specimen collection. Wooden shaft swabs may contain toxins and formaldehydes and should not be used. Polyester-tipped swabs are suitable when specimen collection by a swab is appropriate.
5. Performance Characteristics of Quest V-C-M Medium are validated with BD™ Universal Viral Transport Swabs only. The use of swabs from any other source has not been validated and could affect the performance of the product.

PERFORMANCE CHARACTERISTICS

Viability studies were performed using Quest V-C-M Medium with a variety of viruses, chlamydiae, mycoplasmas and ureaplasmas. Swabs accompanying each transport system were directly inoculated in triplicate with 100 µL of organism suspension. Swabs were then placed in their respective transport medium vials and were held for 0, 24 and 48 h at both 4°C and room temperature (20–25°C). At the appropriate time interval, each swab was vortexed, removed from its transport medium vial and then an aliquot of this suspension was inoculated into shell vials or into appropriate culture media. All cultures were processed by standard laboratory culture technique and examined after a specified incubation time. Organism viability was determined by fluorescing foci counts for viral and chlamydial strains and by CFU counts for mycoplasmal and ureaplasmal strains. Organisms evaluated were: adenovirus, cytomegalovirus, echovirus type 30, herpes simplex virus type 1, herpes simplex virus type 2, influenza A, parainfluenza 3, respiratory syncytial virus, varicella-zoster virus, *Chlamydophila pneumoniae, Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma pneumoniae* and *Ureaplasma urealyticum.*

The results for the strains tested using Quest V-C-M Medium are shown in the following tables.

Quest V-C-M Medium was able to maintain the viability of the following organisms for at least 48 h at both room temperature (20–25°C) and in the refrigerator (2–8°C) under the test conditions described above: adenovirus, cytomegalovirus, echovirus type 30, herpes simplex virus type 1, herpes simplex virus type 2, influenza A, parainfluenza 3, respiratory syncytial virus, varicella-zoster virus, *Chlamydophila pneumoniae, Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma pneumoniae* and *Ureaplasma urealyticum.*

Table 1

Organism	Organism Concentration	Holding Time (hours)	Incubation Time Before Reading (hours)	Viability Challenge at 4°C Foci of infected cells/200 µL[2]	Viability Challenge at RT Foci of infected cells/200 µL[2]
Adenovirus	10^{-1} Neat Virus Stock Suspension* (dilution produces infectivity of 70% of cells)	0 24 48	24 24 24	123 62 68	119 47 63
	10^{-2} Neat Virus Stock Suspension* (dilution produces infectivity of 42% of cells)	0 24 48	24 24 24	17 5 5	14 3 7
Cytomegalovirus	Neat Virus Stock Suspension* (neat produces infectivity of 3% of cells)	0 24 48	24 24 24	337 582 394	444 1012 506
	1:2 Neat Virus Stock Suspension* (dilution produces infectivity of 2% of cells)	0 24 48	24 24 24	49 63 72	195 80 228
Echovirus Type 30	10^{-1} Neat Virus Stock Suspension* (dilution produces infectivity of 64% of cells)	0 24 48	24 24 24	76 59 66	79 75 60
	10^{-2} Neat Virus Stock Suspension* (dilution produces infectivity of 35% of cells)	0 24 48	24 24 24	34 18 25	48 26 20
Herpes Simplex Virus Type 1	10^{-1} Neat Virus Stock Suspension* (dilution produces infectivity of 100% of cells)	0 24 48	24 24 24	491 387 282	412 301 164
	10^{-2} Neat Virus Stock Suspension* (dilution produces infectivity of 100% of cells)	0 24 48	24 24 24	98 68 21	100 10 1
Herpes Simplex Virus Type 2	10^{-1} Neat Virus Stock Suspension* (dilution produces infectivity of 90% of cells)	0 24 48	24 24 24	TNTC[1] 615 525	TNTC[1] 437 58
	10^{-2} Neat Virus Stock Suspension* (dilution produces infectivity of 40% of cells)	0 24 48	24 24 24	228 170 75	315 73 7
Influenza A	Neat Virus Stock Suspension* (neat produces infectivity of 59% of cells)	0 24 48	16 16 16	129 172 166	134 166 169
	10^{-1} Neat Virus Stock Suspension* (dilution produces infectivity of 47% of cells)	0 24 48	16 16 16	123 71 67	115 72 65
Parainfluenza 3	Neat Virus Stock Suspension* (neat produces infectivity of 57% of cells)	0 24 48	24 24 24	24 26 26	32 28 19
	10^{-1} Neat Virus Stock Suspension* (dilution produces infectivity of 51% of cells)	0 24 48	24 24 24	2 12 8	8 10 4
Respiratory Syncytial Virus	Neat Virus Stock Suspension* (neat produces infectivity of 47% of cells)	0 24 48	24 24 24	178 251 183	248 208 232
	10^{-1} Neat Virus Stock Suspension* (dilution produces infectivity of 8% of cells)	0 24 48	24 24 24	17 28 14	13 21 16
Varicella-Zoster Virus	Neat Virus Stock Suspension* (neat produces infectivity of 8% of cells)	0 24 48	72 72 72	TNTC[1] TNTC[1] 283	TNTC[1] TNTC[1] 424
	1:2 Neat Virus Stock Suspension* (dilution produces infectivity of 2% of cells)	0 24 48	72 72 72	TNTC[1] TNTC[1] 132	TNTC[1] TNTC[1] 159

* 100 µL of suspension dosed onto the swab tip then swab placed in V-C-M Medium vial containing 3 mL of transport medium

[1] TNTC= Too numerous to count

[2] Average of triplicate tests performed on 200 µL aliquots of V-C-M Medium at each time point

Table 2

Organism	Organism Concentration	Holding Time (hours)	Incubation Time Before Reading (days)	Viability Challenge at 4°C Fluorescing cytoplasmic inclusions/200 µL[2]	Viability Challenge at RT Fluorescing cytoplasmic inclusions/200 µL[2]
Chlamydophila pneumoniae	Neat *Chlamydophila* Stock Suspension* (neat produces TNTC[1] cytoplasmic inclusions over entire HeLa DHI shell vials coverslip)	0 24 48	3 3 3	TNTC[1] TNTC[1] 201	TNTC[1] TNTC[1] 136
	10^{-1} Neat *Chlamydophila* Stock Suspension* (dilution produces TNTC[1] cytoplasmic inclusions over entire HeLa DHI shell vials coverslip)	0 24 48	3 3 3	256 175 39	257 276 17
Chlamydia trachomatis	Neat *Chlamydia* Stock Suspension* (neat produces TNTC[1] cytoplasmic inclusions over entire BGMK DHI shell vials coverslip)	0 24 48	3 3 3	TNTC[1] TNTC[1] 317	TNTC[1] TNTC[1] 50
	10^{-1} Neat *Chlamydia* Stock Suspension* (dilution produces TNTC[1] cytoplasmic inclusions over entire BGMK DHI shell vials coverslip)	0 24 48	3 3 3	216 164 67	171 48 6

* 100 µL of suspension dosed onto the swab tip then swab placed in V-C-M Medium vial containing 3 mL of transport medium

[1] TNTC= Too numerous to count

[2] Average of triplicate tests performed on 200 µL aliquots of V-C-M Medium at each time point

Table 3

Organism	Organism Concentration	Holding Time (hours)	Incubation Time Before Reading (days)	Viability Challenge at 4°C CFU/200 µL[2]	Viability Challenge at RT CFU/200 µL[2]
Mycoplasma hominis	Neat *Mycoplasma* Stock Suspension*: Four *Mycoplasma hominis* Bacti Disks™ reconstituted into 20 mL of PPLO broth and incubated in 5–10% CO_2 at 35–37°C for 48 h (reference Remel *Mycoplasma* Bacti Disks™ Pack Insert TI No. 19314)	0 24 48	7 7 7	~ 1000, TNTC[1] ~ 1000, TNTC[1] ~ 1000, TNTC[1]	~ 1000, TNTC[1] ~ 1000, TNTC[1] ~ 1000, TNTC[1]
	10^{-2} Neat *Mycoplasma* Stock Suspension*	0 24 48	7 7 7	17 17 11	16 10 12
Mycoplasma pneumoniae	Neat *Mycoplasma* Stock Suspension*: Four *Mycoplasma pneumoniae* Bacti Disks™ reconstituted into 20 mL of SP4 broth with glucose and incubated in ambient air at 35–37°C for 7–14 days until broth becomes yellow (reference Remel *Mycoplasma* Bacti Disks™ Pack Insert TI No. 19314)	0 24 48	7 7 7	171 219 183	169 238 184
	10^{-1} Neat *Mycoplasma* Stock Suspension*	0 24 48	7 7 7	17 22 17	18 26 19
Ureaplasma urealyticum	Neat *Ureaplasma* Stock Suspension*: Ten *Ureaplasma urealyticum* Bacti Disks™ reconstituted into 18 mL of 10B broth and incubated in ambient air at 35–37°C for 24 h (reference Remel *Ureaplasma* Bacti Disks™ Pack Insert TI No. 19315)	0 24 48	3 3 3	1020 1136 1249	1125 1083 1056
	10^{-1} Neat *Ureaplasma* Stock Suspension*	0 24 48	3 3 3	101 107 116	83 108 103

* 100 µL of suspension dosed onto the swab tip then swab placed in V-C-M Medium vial containing 3 mL of transport medium

[1] TNTC= Too numerous to count

[2] Average of triplicate tests performed on 200 µL aliquots of V-C-M Medium at each time point

AVAILABILITY

Cat. No.	Description
220223	Quest V-C-M Medium 3 mL Vial, carton of 300.

REFERENCES

1. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
2. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. *17*: 53-80.
3. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
4. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken. 2003. Manual of clinical microbiology. 8th ed. ASM, Washington, D.C.
6. Gleaves, C.A., R.L. Hodinka, S.L.G. Johnston, and E.M. Swierkosz. 1994. Cumitech 15A. Laboratory diagnosis of viral infections. ASM, Washington, DC.
7. Forbes, B.A., D.F. Sahm, and A.S Weissfeld. 2002. Bailey and Scott's diagnostic microbiology. 11th ed. Mosby, St. Louis, MO.
8. Wardford, A., M. Chernesky, and E. M. Peterson. 1999. Cumitech 19A, Laboratory diagnosis of *Chlamydia trachomatis* infections. ASM, Washington, DC.
9. Miller, J. M. 1999. A guide to specimen management in clinical microbiology, 2nd ed. ASM, Washington, DC.
10. Isenberg, H. D., 2004. Clinical microbiology procedures handbook, 2nd ed. ASM, Washington, DC.
11. Isenberg, H.D., 1998. Essential procedures for clinical microbiology. Chapter 14.12, Page 787. Packaging and shipping infectious substances.
12. Maass, M. and K. Dalhoff. 1995. Transport and storage conditions for cultural recovery of *Chlamydia pneumoniae*. J. Clin. Microbiol. *33*:1793-1796.
13. Johnson, F. 2005. Transport of viral specimens. Clin. Microbiol. Rev *3*:120-121.
14. 42CFR72. Code of Federal Regulations, Title 42, Volume 1, Part 72. Interstate Shipment of Etiologic Agents.
15. National Committee for Clinical Laboratory Standards (NCCLS). 1994. Procedures for handling and transport of diagnostic specimens and etiologic agents. Approved Standard H5-A3, NCCLS, Wayne, PA.
16. Clinical and Laboratory Standards Institute. 2003. Approved Standard M40-A. Quality control of microbiological transport systems. CLSI, Wayne, PA.
17. Clinical and Laboratory Standards Institute. 2006. Approved Guideline M41-A Viral culture. CLSI, Wayne, PA.

Distributed by

Becton, Dickinson and Company
Made in Italy by Copan