



INSTRUCTIONS FOR USE

CHOCOLATE AGAR (CHOC)

PRODUCTS

AS-214 Chocolate Agar (CHOC)	1 plate / pkg
AS-244 Chocolate Agar (CHOC)	4 plates / pkg

INTENDED PURPOSE

TruPRAS™ Anaerobic Culture Media is intended for the transport, preservation, or cultivation of a wide variety of microorganisms from specimens to aid in the isolation of bacteria for in vitro diagnostic and / or research / general laboratory purposes.

INTENDED USERS

Scientists, laboratory, and healthcare professionals trained in anaerobic microbiology techniques working in areas such as clinical, research, industrial, pharmaceutical and veterinary applications.

FORMULATION*

CHOC is an enriched, non-selective medium recommended for the growth of fastidious aerobic and anaerobic organisms. It supports the growth of pathogenic bacteria, including fastidious respiratory pathogens such as *Haemophilus influenzae* and *Neisseria meningitidis*. CHOC agar is supplemented with cofactors V (nicotinamide adenine dinucleotide, NAD) and X (hemin), which facilitate the growth of fastidious organisms, particularly those requiring these factors for metabolism. Heated horse blood is added to the medium, giving it a characteristic "chocolate" color. This blood has been lysed by slowly heating to 80°C, releasing intracellular nutrients like NAD and hemin, which are essential for the growth of certain bacteria. The heating process also inactivates enzymes that could otherwise degrade NAD. CHOC agar is recommended for aerotolerance testing of obligate anaerobes. Despite its name, the medium contains no chocolate products, the name refers solely to its distinctive color. This medium is prepared, dispensed, and packaged under oxygen-free conditions using TruPRAS™ Technology to prevent the formation of oxidized products prior to use. This product is supplied ready to use, with no pre-reduction step required.

Pancreatic digest of casein	10.00	g
Soy peptone	3.00	g
Meat peptone	10.00	g
Dextrose	1.00	g
Yeast extract	2.00	g
Sodium chloride	5.00	g
Sodium bisulfite	0.10	g
Agar	15.00	g
VX supplementation	10.00	mL
Defibrinated horse blood	50.00	mL
DI Water	1.00	L

*Approximate formula. Adjusted and/or supplemented as required to meet performance criteria.

Final pH: 7.0 ± 0.2 at 25°C

Final weight: 16.0 g ± 1.6 g



PRECAUTIONS

For IN VITRO DIAGNOSTIC USE only. Utilize approved biohazard precautions and aseptic technique when using this product. This product is for use by properly trained and qualified personnel only. Sterilize all biohazard waste prior to disposal. This product is manufactured as a single use device.

Report serious incidents that occur in direct relation to this product to tech@biolog.com. As necessary, report serious incidents to the regulatory authority in which the user is established.

This product may contain components of animal origin. All components of animal origin have been sourced from Bovine Spongiform Encephalopathy- (BSE-) free and Transmissible Spongiform Encephalopathy- (TSE-) free countries. Certified knowledge of the origin of animal derived components does not guarantee the absence of transmissible pathogenic agents. It is recommended that Universal Precautions be observed.

When working with anaerobic culture media, the potential for ergonomic hazards may exist due to repetitive motions, awkward postures, improper bench/chair heights or poor lighting. Although it is beyond the scope and provision of products by Anaerobe Systems, it should be recognized and mitigated by the end user in the laboratory environment.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2 – 8°C in original package until used. Avoid overheating or freezing. Do not use media if there are signs of deterioration (shrinking, cracking, or discoloration due to oxidation of media) or contamination. The expiration date applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the label.

Shelf Life: 9 months from the date of manufacture.

PROCEDURE

Specimen Collection: Protect specimens for anaerobic culture from oxygen during collection, transportation, and processing. Consult appropriate references for detailed instructions concerning collection and transportation of anaerobes. The selection of specimens for culture is made by physicians or scientists collecting the sample.

Methods for Use: CHOC agar should be inoculated directly with a specimen or from a broth previously inoculated with a specimen. Plates should be incubated in 5 – 10% CO₂ for 24 to 48 hours, particularly when performing aerotolerance testing. The CHOC agar surface can be divided to allow isolation of multiple organisms on a single plate. It is recommended to inoculate CHOC agar first to ensure that any “no growth” result reflects atmospheric requirements rather than insufficient inoculum. CHOC agar is preferred over standard blood agar plates for detecting *Haemophilus influenzae* and other fastidious microorganisms that can grow anaerobically and might be missed in aerotolerance tests conducted solely on blood agar. Extended incubation periods may be necessary to recover certain slow-growing organisms. For detailed instructions on processing aerobic and anaerobic cultures, please refer to the appropriate references. As packaged, this medium constitutes a qualitative, manual method.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, disinfectant, other culture media, and serological / biochemical reagents.

INTERPRETATION OF RESULTS

CHOC should support robust growth of most clinically relevant pathogens, including *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and other fastidious aerobic and anaerobic organisms under appropriate environmental conditions.



Microorganisms that grow on CHOC agar when incubated in 5 – 10% CO₂ are not obligate anaerobes. Therefore, findings should correlate with organisms identified in the aerobic culture and the direct Gram stain.

LIMITATIONS

CHOC agar does not provide complete information for the identification of bacterial isolates. Additional test procedures and media are required for full identification. It is important to note that some anaerobic microorganisms can be aerotolerant, including certain species of *Clostridium*, *Actinomyces*, *Cutibacterium*, *Bifidobacterium*, and *Lactobacillus*. These organisms typically grow well under anaerobic conditions but may grow poorly in the presence of carbon dioxide or ambient air. Refer to appropriate reference materials for additional guidance.

QUALITY CONTROL

The following organisms are routinely used for quality control testing at Anaerobe Systems using the specifications outlined in the CLSI document M22-A3: Quality Control for Commercially Prepared Microbiological Culture Media.

Organism Tested	ATCC® #	Results in 5% CO ₂	Results in anaerobic conditions
<i>Fusobacterium necrophorum</i>	25286	No growth	Growth
<i>Neisseria gonorrhoeae</i> *	43069	Growth	Poor growth
<i>Haemophilus influenzae</i> *	10211	Growth	Growth
<i>Streptococcus pneumoniae</i>	49619	Growth	Growth
<i>Staphylococcus aureus</i>	25923	Growth	Growth
<i>Escherichia coli</i>	25922	Growth	Growth

* Organisms recommended by CLSI for quality control testing of Chocolate agar.

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If the nutritive capacity of this medium is to be tested for performance, it is recommended that the following ATCC® organisms be evaluated for growth.

Organism	ATCC® #	Expected Results
<i>Fusobacterium necrophorum</i>	25286	Growth*
<i>Neisseria gonorrhoeae</i>	43069	Growth
<i>Haemophilus influenzae</i>	10211	Growth
<i>Streptococcus pneumoniae</i>	49619	Growth

* Growth in anaerobic conditions only.

Physical Appearance: CHOC agar should appear opaque and milk chocolate brown in color.

ATCC® is a registered trademark of American Type Culture Collection.

REFERENCES

1. CLSI. *Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline*. CLSI document M56-A. Clinical and Laboratory Standards Institute; 2014
2. Leber AL, Burnham CA, eds. *Clinical Microbiology Procedures Handbook*. 5th ed. 4 vols. Washington, DC: ASM Press; 2023.



3. Carroll KC, Pfaller MA, eds. *Manual of Clinical Microbiology*. 13th ed. 4 vols. Hoboken, NJ: Wiley-Blackwell; 2023.
4. Jousimies-Somer HR, Sutter VL, eds. *Wadsworth-KTL Anaerobic Bacteriology Manual*. 6th ed. Belmont, CA: Star Publishing Company; 2002.
5. CLSI. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard- Third Edition*. CLSI document M22-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.
6. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. *Animal Health Status of Regions*. Published March 12, 2025. <https://www.aphis.usda.gov/regionalization-evaluation-services/region-health-status>
7. European Commission. *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 Rev. 3)*. Published March 5, 2011. <https://op.europa.eu/en/publication-detail/-/publication/3392e464-ba89-4ae4-955c-a07f617c8e06/language-en>

GLOSSARY OF SYMBOLS

SYMBOL	TITLE	DESCRIPTION	STANDARD	REF#
	Catalog number	Indicates the manufacturer's catalog number so that the medical device can be identified.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.1
	Lot number/ Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.5
	Use-by date	Indicates the date after which the medical device is not to be used.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.4
	Authorized Representative	Indicates the Authorized Representative in the identified country or jurisdiction.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.2
	Do not re-use/ Single use only	Indicates a medical device that is intended for one single use only.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.4.2
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.4.3
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.3.7
	In vitro diagnostic medical device	Indicates that a medical device is intended to be used as an in vitro diagnostic medical device	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.5.1
	CE Mark European Conformity	Designates that the product labeled is authorized for sale in European countries.	EU IVDR (EU) 2017/746	

AUTHORIZED REPRESENTATIVE INFORMATION



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REVISION 5

Additions: None

Changes: Extended shelf life from 6 months to 9 months.

Deletions: None