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Viral Culture; Approved Guideline

This document provides guidance for viral culture and identification procedures performed in the clinical virology laboratory.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Abstract

This document provides guidance for viral culture and identification procedures that are typically performed in the clinical virology laboratory setting using commercially available cell cultures and reagents. The nature of the cell culture system is one that is inherently variable and thus remains susceptible to numerous adverse conditions that can lead to unreliable results. Several critical elements that must be addressed in devising a viral culture procedure are identified. These include: cell culture selection, assessment and maintenance; cell culture verification and quality control; culture medium preparation and quality control; specimen collection and preparation; isolate identification; and result reporting and interpretation. The intended audience includes laboratories performing either limited or comprehensive viral cultures as well as those that are considering introduction of viral culture. Regardless of the viral diagnostic testing algorithm utilized by a laboratory, the basic principles of viral culture are universal.

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Foreword

This document provides guidance for viral culture and identification procedures that are typically performed in the clinical virology laboratory setting using commercially available reagents and monolayered cell cultures and does not include procedures for isolation of agents requiring specialized cell culture systems or procedures for their cultivation. The nature of the cell culture system is one that is inherently variable and thus remains susceptible to numerous adverse conditions that can lead to unreliable results. Given this, and the number of other variables associated with culture procedures, it would be impossible to identify a consensus guideline that would not be restrictive. This document is intended to identify the many variables associated with viral culture procedures and to provide guidance regarding several critical elements that must be addressed in devising a viral culture procedure. The critical elements of a viral culture procedure include specimen collection, processing, and inoculation; cell culture selection, assessment, maintenance, and quality control; isolate detection and identification; and reporting and interpretation of test results, including information regarding potential limitations of the procedure.

This document includes procedures and guidance that are appropriate for different laboratory settings ranging from those offering a limited culture menu (e.g., herpes simplex virus [HSV]), to those performing more comprehensive viral culture. The intended audience includes not only laboratories already performing viral culture but also those that may have hesitated to introduce viral culture because of perceived difficulties of the method. This document also includes cautionary notes related to recent safety concerns regarding BSL-3 and BSL-4 agents that have been identified as either potential biologic threats (e.g., variola) or emergent infections (e.g., severe acute respiratory syndrome – coronavirus [SARS-CoV], avian influenza).

Key Words

Cell culture, viral culture, viral isolation

Viral Culture; Approved Guideline

1 Scope

This document focuses on viral culture and identification procedures that are typically performed in the clinical virology laboratory setting using commercially available monolayered cell cultures and reagents. Guidance for specimen collection, processing, and inoculation; cell culture selection, assessment, maintenance, and quality control; isolate detection and identification; and reporting and interpretation of test results, including information regarding potential limitations of the procedure, are outlined. The intended audience includes laboratories performing either limited or comprehensive viral culture procedures, as well as those that are considering introduction of viral culture.

2 Introduction

Comprehensive diagnostic virology laboratories typically utilize a combination of culture and nonculture techniques for the detection of viral agents in clinical samples. Historically, culture methods were considered cumbersome and complex, with turnaround times that precluded their clinical utility. In addition, many microbiology laboratories have hesitated to incorporate viral culture into their test menus because of the perceived difficulties associated with handling cell cultures and identifying viral isolates. However, the commercial availability of high quality cell cultures, culture media, and culture confirmation reagents has served to expand the availability of viral cultures.

The nature of the cell culture system is one that is inherently variable and thus remains susceptible to numerous adverse conditions that can lead to unreliable results. In addition, a viral cell culture procedure may vary depending on the needs of a particular setting and the availability of complementary procedures. These variables underscore the need to develop a procedure that adequately addresses the critical elements necessary for performing a reliable viral culture procedure. This guidance document is thus intended to provide recommendations for optimizing culture results. This document also includes cautionary notes related to recent safety concerns regarding BSL-3 and BSL-4 agents that have been identified as either potential biologic threats (e.g., variola) or emergent infections (e.g., SARS-CoV, avian influenza), and that can replicate in cell lines typically employed by the diagnostic laboratory.

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (Garner JS, Hospital Infection Control Practices Advisory Committee. *Guideline for Isolation Precautions in Hospitals.* Infection Control and Hospital Epidemiology. CDC. 1996;17(1):53-80). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections*. Categorization of biologic agents according to their biosafety level (BSL) and a detailed description of recommended facilities, practices, and protective equipment for the various levels are available at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm (Public Health Service, CDC and NIH. *Biosafety*

in Microbiological and Biomedical Laboratories, 4th ed. Washington DC: United States Government Printing Office; 1999).

4 Definitions

blind subpassage – transfer of cells and/or medium from an existing viral culture to a fresh cell culture monolayer(s).

cell culture propagation – serial transfer of suspended cell culture cells (e.g., derived from a trypsinized monolayer) to a fresh culture vessel containing growth medium.

cytopathic effect (CPE) – a variety of morphologic changes occurring in monolayered cell cultures as a result of viral infection.

growth medium – cell culture nutrient solution intended to promote adhesion of dispersed (e.g., trypsinized) cells to a culture vessel surface and/or to support mitotic division of cells.

hemadsorption (HAd) – adherence of certain red blood cells to the surface of monolayered cells; **NOTE:** HAd is mediated by expression of viral hemagglutinin proteins on the surface of cells infected by certain viruses (e.g., influenza) and can occur in the absence of CPE.

hemagglutination – *in viral culture*, the clumping of certain red blood cells that can be observed in culture supernatants containing hemagglutinin proteins shed by cells infected by certain viruses (e.g., influenza).

maintenance medium – cell culture nutrient solution intended to maintain viability and integrity of a confluent or nearly confluent cell culture monolayer without promoting abundant mitotic division.

monolayered cell culture – *in vitro* culture consisting of a single layer of cells (epithelial-like or fibroblast) that are adherent to the surface of a culture vessel; **NOTE:** The degree of monolayer confluence refers to the percentage of the culture vessel's growth surface that is populated with cells. For example, 50% confluence would indicate that the viable adherent cells of the developing monolayer occupy approximately half of the growth surface area. A monolayer at 100% confluence is one in which a sheet of contiguous cells covers the entire growth surface.

senescent cell culture – culture with reduced or arrested metabolic and growth activity.

shell vial – flat-bottomed glass vial suitable for holding a coverslip on which the cell monolayer develops.

syncytium – a type of CPE resulting from the fusion of adjacent cells; **NOTE:** Also referred to as a multinucleated giant cell.

titration – a method used to determine the infectivity titer of a viral preparation; **NOTE 1:** Serial dilutions (e.g., twofold or tenfold) are prepared in diluent (e.g., balanced salt solution [BSS]) and inoculated into replicate (e.g., 6 to 8) cell cultures; **NOTE 2:** The tissue culture infectious dose ($TCID_{50}$) is the dilution of virus that infects 50% of the monolayers; **NOTE 3:** The $TCID_{50}$ is considered the endpoint dilution, and the endpoint titer is the inverse of the dilution.

toxicity – morphologic changes and/or cell lysis occurring in monolayered cell cultures induced by the presence of toxins, pH extremes, metabolites, or other chemical compounds.

Quality System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.* The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any healthcare service's path of workflow (i.e., operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

M41-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X						

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M41-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other Clinical and Laboratory Standards Institute documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
	X	X	X	X	X	X	X	X

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Related CLSI/NCCLS Publication*

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most recent editions.

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