

Contents

	Foreword
	Introduction
1	Scope
2	Normative references
3	Terms and definitions
4	Materials
4.1	Virus and host cell to be used for the tests
4.2	Reagents
4.3	Culture medium and solutions
4.3.1	General
4.3.2	Eagle's minimum essential medium (EMEM)
4.3.3	RPMI 1640 medium
4.3.4	7,5 % sodium bicarbonate solution
4.3.5	Formaldehyde solution
4.3.6	Methylene blue solution
4.3.7	Inactivated fetal bovine serum (FBS)
4.3.8	Growth medium
4.3.9	Maintenance medium
4.3.10	Double concentration of the maintenance medium
4.3.11	Phosphate buffered saline [PBS (-)]
4.3.12	Trypsin derived from beef pancreas and PBS (-) solution
4.3.13	Trypsin EDTA solution ¹ 1 Trypsin EDTA solution is an example of a product available in the market. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.
4.3.14	DEAE-dextran solution
4.3.15	Agar medium for plaque assay
4.3.15.1	A liquid
4.3.15.2	B liquid
4.3.15.3	Preparation of agar medium
4.3.16	Soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate (SCDLP broth)
5	Apparatus
6	Preparation
6.1	Restoration of host cell from cryopreservation
6.2	Subculture of host cell
6.3	Cell culture for measuring virus infectivity titer
6.4	Preparation of test inoculums
6.4.1	Influenza virus
6.4.2	Feline calicivirus
6.5	Preparation of test specimens
6.6	Control test
6.6.1	General
6.6.2	Verification of cytotoxic effect on host cell
6.6.3	Verification of cell sensitivity to virus and the inactivation of antiviral activity
6.6.3.1	Procedure for verification
6.6.3.2	Determination of the infectivity titer of virus
6.6.3.3	Condition for verification for this test

- 7 **Test procedure**
 - 7.1 Preparation of test specimen
 - 7.2 Inoculation of test specimens
 - 7.3 Contact of the inoculated test specimens
 - 7.4 Recovery of virus from test specimens
 - 7.4.1 Test specimens immediately after inoculation
 - 7.4.2 Test specimens after contact
 - 7.5 Determining the infectivity titer of virus by plaque assay
- 8 **Expression of results**
 - 8.1 Determination of the infectivity titer of virus
 - 8.2 Conditions for a valid test
 - 8.3 Calculation of the antiviral activity
 - 8.4 Effectiveness of the antiviral agent
- 9 **Repeatability and reproducibility**
- 10 **Test report**
- Annex A (informative) Composition of media**
 - A.1 General
 - A.2 Composition of EMEM
 - A.3 Composition of RPMI 1640 medium
- Annex B (informative) Repeatability and reproducibility**
 - B.1 Background
 - B.2 Experiment
 - B.3 Results and discussion

Page count: 20