National Institute of Allergy and Infectious Diseases

Overview of Routine Tests for Adventitious Agents

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Outline

- Potential Sources of Contamination (need for Adventitious Agent Tests)
- Routine Tests
- In Vitro Tests
- In Vivo Tests
- Retrovirus Tests
- Specific virus tests
- Bovine Spongiform Encephalopathy (BSE)

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Potential Sources of Contamination

- Cell Substrate
  - Viral Seed
  - Cell Substrate(s) used in passage history of Viral seed
- Animal
  - Animal-derived or human-derived materials used in culture (passage history or production) or downstream processing (e.g., albumin added as virus stabilizer)
- Personnel
- Facility
- Other production materials (flasks, etc.)

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Adventitious Agent Testing

- Bacterial and fungal sterility (610.12)
- Mycoplasma (& spiroplasma)
  - cultivable, non-cultivable
- Mycobacteria (animals/culture-650.13)
- Viruses (*in vitro*, *in vivo*)
  - Acute (Lytic, Hemadsorbing/Hemagglutinating)
  - Latent (e.g., retroviruses or other oncogenic viruses)
- Animal-derived raw materials
  - 9 CFR 113 tests
  - from BSE-free countries

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Breadth & Sensitivity

- These tests were developed for clinical diagnostics in mid-1900's
- Initially, they were used to detect SPECIFIC adventitious agents
- Their use was expanded to be broad general screening assays
- Their breadth/sensitivity has not been systematically assessed
- They have not been validated in the manner currently developed assays would be required to be validated

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Adventitious Agent Test Methods

- *In Vitro* tests
  - monolayers of at least 3 cell types
    - same species, tissue as substrate
    - human diploid cells
    - monkey kidney cells
  - tests for hemadsorption and hemagglutination at end of culture period
- *In Vivo* tests
  - adult and suckling mice
  - embryonated hens' eggs
  - when appropriate
    - guinea pigs
    - rabbits

Tissue Cultures

- Monkey Kidney Cells (originally to detect simian agents)
- Human Diploid Cells (originally to detect measles)
- Same species, tissue as production
- 14 days, subculture for additional 14 days
- CPE, hemadsorption/hemagglutination (RBCs of guinea pigs, chickens, and human or rhesus monkey – 2 temperatures, ½ hr each)
- Capable of detecting a wide array of human and other viruses
- Larger sample volume (3 mL/culture) applied than delivered in the in vivo tests

Adult Mice

- LCMV or other viruses
- ≥20 adult mice
- i.p. with 0.5 mL, i.c., with 0.03 mL
- Mice must survive 21 days
  - ≥80% survival
  - No sign of viral infection
- Capable to detect LCMV, coxsackieviruses, flaviviruses, rabies

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Suckling Mice

- Coxsackieviruses (particularly type A)
- ≥20 mice less than 24 hours old
- i.c., 0.01 mL, i.p., 0.1 mL
- 14 days
- Subinoculation into additional mice for 14 days
- Mice must survive
  - ≥80% survival both inoculations
  - No signs of viral infection
- Capable of detecting coxsackieviruses, other picornaviruses (polioviruses, echoviruses), alphaviruses, herpesviruses (HSV), flaviviruses, rabies, many murine agents, others

Embryonated Hens' Eggs

- 10-11 day-old embryos, 0.5 mL allantoic route, 3 days, HA
  - Orthomyxoviruses (influenzaviruses), paramyxoviruses (mumps, measles, parainfluenzaviruses), alphaviruses
- 6-7 day-old embryos, 0.5 mL yolk sac route, ≥9 days, survival
  - Herpesviruses (HSV), poxviruses, rhabdoviruses, rickettsiae, mycoplasmas, bacteria

Guinea Pigs

- LCMV, M. Tb
- ≥5 guinea pigs
- 0.1 mL i.c., 5 mL i.p.
- 42 days
- ≥80% survival
  - No signs of LCMV or M. Tb infection
- Capable of detecting paramyxoviruses, reoviruses, filoviruses

Rabbits

- Used to detect B virus – methods similar to guinea pig test (i.e., 5 rabbits)
- Not used routinely

Antibody Production

- For rodent cell substrates or materials exposed to rodent-derived raw materials (e.g., antisera used for affinity chromatography or selection pressure)
  - Mouse (MAP)
  - Rat (RAP)
  - Hamster (HAP)
    - Viruses specified
    - Animals exposed
    - Sera collected for antibody production assay
  - Live LCMV challenge
    - Because some strains of LCMV may not result in apparent infection, if animal develops antibodies to LCMV contaminant and are challenged with virulent LCMV, they will be protected

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Retrovirus Tests

- Reverse Transcriptase
  - PERT
- Transmission Electron Microscopy
- Infectivity Assays
  - Particularly for rodent cell substrates

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Specific Viruses

- Other in vitro methods are used to detect specific viruses
  - Usually PCR/NAAT methods
  - List of specific viruses depends on species and donor medical history
  - Human Viruses
    - HIV-1, HIV-2, HTLV-1, HTLV-2 (also detectable by PERT)
    - HAV, HBV, HCV
    - CMV, EBV
    - HHV-6, HHV-7, HHV-8
    - AAV
  - Simian Viruses – SV40, sCMV
  - List will continually grow

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Bovine and Porcine Viruses

- 9 CFR 113 methods
  - Exposure of test material to Vero cells and either bovine turbinate or porcine testes (or similar) cells
  - Specific incubation period
  - Cytopathic effect (with histological staining)
  - Hemadsorption with guinea pig and chicken RBCs at specific temperatures/times of incubation
  - Immunofluorescent antibody staining for specific viruses

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Bovine Spongiform Encepalopathies

- Biologicals cannot be tested for BSE/TSE (currently)
- Potential exposure must be controlled through traceability of source materials
  - Monitoring of source herds
  - Geographic sourcing
  - Removal of high-risk materials/abattoir practices
- Minimization of exposure to animal-derived materials
  - Animal-protein-free or serum-free raw materials
  - Recombinant or plant-derived materials

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Summary

- The routine adventitious agent tests list has grown over the decades as it has become recognized that many viral families are not detectable by the methods originally promulgated in the 1960's
- New methodologies are emerging that have the potential to either replace or permit refinement of the routine methods
- The new methods will be held to standards of assay validation that the oldest routine methods have not
- Comparison of new methods to old methods will be complicated by this lack of baseline data on the old methods
- Adoption of new methods as replacements may require establishing that they are equivalent or better (21 CFR 610.9)

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