Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Inactivation and Stability of Viral Diagnostic Reagents Treated by Gamma Radiation

L. A. White,* C. Y. Freeman,t H. E. Hall† and B. D. Forrester††

Scientific Resources Program* and Division of Viral Diseases,† Centre for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333 U.S.A.

Abstract. The objective of this study was to apply the pertinent findings from gamma inactivation of virus infectivity to the production of high quality diagnostic reagents. A Gammacell 220 (Atomic Energy of Canada, Ltd., Ottawa, Canada$) was used to subject 38 viruses grown in either susceptible tissue cultures or embryonated chicken eggs to various doses of gamma radiation from a cobalt-60 source. The radiation required to reduce viral infectivity was 0.42 to 3.7 megarads (Mrad). The effect of gamma treatment on the antigenic reactivity of reagents for the complement fixation (CF), hemagglutination (HA) and neuraminidase assays was determined. Influenza antigens inactivated with 1.7 Mrad displayed comparable potency, sensitivity, specificity and stability to those inactivated by standard procedures with beta-propiolactone (BPL). Significant inactivation of influenza N1 and B neuraminidase occurred with >2.4 Mrad radiation at temperatures above 4°C. All 38 viruses were inactivated, and CF or HA antigens were prepared successfully. Antigenic potency remained stable with all antigens for 3 years and with 83% after 5 years storage. Influenza HA antigens evaluated after 9 years of storage demonstrated 86% stability. Gamma radiation is safer than chemical inactivation procedures and is a reliable and effective replacement for BPL in preparing diagnostic reagents.

Introduction

Gamma radiation has been used to inactivate human and animal viruses1-4 and diagnostic biological products.5-8 This method has been described for a wide range of uses including sterilizing raw and cooked ground beef containing Coxsackievirus B-29 and for sterilizing laboratory effluent before its release into the environment.10 Large pools of animal serum used in tissue culture virus medium has been radiation treated to inactivate adventitious viruses (HyClone Laboratories, Inc., Logan, Utah, U.S.A.).

The Centers for Disease Control (CDC) prepares diagnostic and reference reagents for many viral diseases of public health importance.11 Reference reagents are supplied to state, county and World Health Organization laboratories as well as commercial manufacturers of diagnostic reagents. Because of the potential hazard of shipping infectious materials, CDC supplies only inactivated reagents. Historically, biologicals have been inactivated by treatment with heat, ultraviolet light and various chemicals including beta-propiolactone (BPL). Because of the potential carcinogenic hazard to the laboratorian associated with the use of BPL12 and the deleterious effects of BPL on some hemagglutinating (HA) antigens, our laboratory investigated inactivation of viral reagents by gamma radiation from a cobalt-60 source. Gamma radiation offers a low energy, non-polluting method of killing viruses under controlled conditions. The resulting product is safe and radioactively inert. This report establishes the dosage levels required for inactivation and treatment of viral diagnostic reagents and discusses the long-term stability of those reagents.

Material and methods

Gamma radiation source

The gamma radiation source was a Gammacell 220 (Atomic Energy of Canada, Ltd., Ottawa, Canada) containing cobalt-60 with an initial activity of 1.96 × 10⁶ rad/h or 1.96 megarad/h (Mrad). Calculations were made at the time of use to account for the decay of the cobalt 60 source.
Table 1. Virus strains and the host systems used for infectivity inactivation studies and for CF and HAI reagent production

| Virus               | Strain | Culture host* | Maintenance medium† | Diagnostic antigens‡ |
|---------------------|--------|---------------|---------------------|----------------------|
| Coronavirus         | 229E   | HEL           | EMEM + 2% FBS       | CF                   |
| Cytomegalovirus     | AD-169 | HEL           | EMEM + 2% FBS       | CF                   |
| Enteroviruses       |        |               |                     |                      |
| Coxackie A-4        | High Point | RD         | EMEM + 2% FBS       | CF                   |
| Coxackie A-24       | Singapore | HEL         | EMEM + 2% FBS       | CF                   |
| Coxackie D-5        | Faulkner | LLC-MK₂     | EMEM + 2% FBS       | CF                   |
| Echovirus 9         | Hill   | LLC-MK₂       | EMEM + 2% FBS       | CF                   |
| Enterovirus 68      | Fermon | HRL          | EMEM + 2% FBS       | CF                   |
| Enterovirus 69      | Toluca 1 | HEL         | EMEM + 2% FBS       | CF                   |
| Enterovirus 70      | J670/7 | RD            | EMEM + 2% FBS       | CF                   |
| Enterovirus 71      | BrCr   | RD            | EMEM + 2% FBS       | CF                   |
| Poliovirus 1        | Mahoney | RD            | EMEM + 2% FBS       | CF                   |
| Poliovirus 2        | Slater | LLC-MK₂      | EMEM + 2% FBS       | CF                   |
| Poliovirus 3        | Saukett | RDF          | EMEM + 2% FBS       | CF                   |
| Herpesvirus         |        |               |                     |                      |
| Herpes Type 1       | MacIntyre | VERO     | Richter + 1% FBS   | CF                   |
| Herpes Type 2       | MS     | VERO          | Richter + 1% FBS   | CF                   |
| Influenzaviruses    |        |               |                     |                      |
| A/PR/8/34(H1N1)     |        | Egg/Alf       |                     | HAI                  |
| A/USSR/90/77(H1N1)  |        | Egg/Alf       |                     | HAI                  |
| A/Brazil/11/78(H1N1) |       | Egg/Alf       |                     | HAI                  |
| A/Japan/170/62(H2N2) |       | Egg/Alf       |                     | HAI                  |
| A/Hong Kong/8/68(H3N2) |      | Egg/Alf       |                     | HAI                  |
| A/Victoria/3/75(H3N2) |     | Egg/Alf       |                     | HAI                  |
| A/Texas/1/77(H3N2)  |        | Egg/Alf       |                     | HAI                  |
| A/Equine/Praque/56  |        | Egg/Alf       |                     | HAI                  |
| (Heq1Neq1)          |        | Egg/Alf       |                     | HAI                  |
| A/Equine/Miami/63   |        | Egg/Alf       |                     | HAI                  |
| (Heq2Neq2)          |        | Egg/Alf       |                     | HAI                  |
| A/New Jersey/8/76   |        | Egg/Alf       |                     | HAI                  |
| (Hsw1Ne1)           |        | Egg/Alf       |                     | HAI                  |
| B/Lee/40            |        | Egg/Alf       |                     | HAI                  |
| B/Hong Kong/5/72    |        | Egg/Alf       |                     | HAI                  |
| C/Taylor/1339/47    |        | Egg/Amn       |                     | HAI                  |
| Mobillivirus         |        |               |                     |                      |
| Measles             | Phila/26 | HEL         | EMEM + 2% FBS       | CF                   |
| Paramyxoviruses     |        |               |                     |                      |
| Type 1              | C-39   | PrRrhK        | EMEM + 2% FBS       | CF                   |
| Type 3              | C243   | VERO          | EMEM + 2% FBS       | CF                   |
| Newcastle Disease   | Roakin | Egg/Alf       |                     | HAI                  |
| Pneumovirus         |        |               |                     |                      |
| Respiratory Syncytial |    | Long         | VERO                | Richter + 1% FBS   | CF                   |
| Reoviruses          |        |               |                     |                      |
| Type 1              | Lang   | LLC-MK₂       | EMEM + 2% FBS       | HAI                  |
| Type 2              | Jones  | LLC-MK₂       | EMEM + 2% FBS       | HAI                  |
| Type 3              | Dearing | LLC-MK₂     | EMEM + 2% FBS       | HAI                  |
| Rotaviruses         |        |               |                     |                      |
| Calf                | NCDV   | MA-104        | M199 wo serum       | CF                   |
| Simian              | SA-11  | MA-104        | M199 wo serum       | CF                   |
| Varicellavirus      | VZ-10  | HEL           | EMEM + 2% FBS       | CF                   |

* Culture hosts: HEL, human embryonic lung; RD, human rhabdomyosarcoma; LLC-MK₂, rhesus monkey kidney; PrRrhk, primary rhesus monkey kidney; VERO, African green monkey kidney; MA-104, African green monkey kidney cell cultures; Egg/Alf, embryonated chicken egg allantoic fluid; and Egg/Amn, embryonated chicken egg amniotic fluid.
† Maintenance medium: EMEM + 2% FBS, Eagle minimum essential medium plus 2% fetal bovine serum, V/V; Richter synthetic medium plus 1% FBS, V/V; EMEM + 5% Chs, EMEM plus 5% chicken serum, V/V; and M199 wo serum, Medium 199 without serum.
‡ Diagnostic antigens: CF, complement fixation; and HAI, hemagglutination antigens.
Virus strains

Thirty-eight reference virus strains (Table 1), comprising 11 genera in seven families, were used in these studies. Each virus was grown in the host system giving maximum sensitivity to infection and was used routinely in reagent production. All viruses were passed in the laboratory at least twice before use as inocula for reagent preparation.

Virus cultures

Six cell lines (Table 1) were used as the routine culture systems for producing diagnostic reagents of the viruses under examination. These cell lines were human embryonic lung (HEL), human rhabdomyosarcoma (RD), rhesus monkey kidney (LLC-MK2), primary rhesus monkey kidney (PrRhK), African green monkey kidney (VERO 76), and African green monkey kidney (MA-104).

Cell monolayers were grown either on 150 cm² culture flasks or on 850 cm² roller bottles. When growth of the cell monolayer was confluent, the growth medium was removed, and the monolayer was rinsed with serum-free medium. The viral inoculum was added to the monolayer and adsorbed to the cells for 2 h at 37°C before the maintenance medium (Table 1) was added. Cultures were incubated at 37°C and harvested when maximum cytopathic effect (CPE) was observed.

Influenza and Newcastle Disease viruses were prepared in 10-day-old embryonated chicken eggs inoculated by the allantoic route (Egg/Ah). Influenza type C virus was cultured in embryonated eggs inoculated by the amniotic route (Egg/Amn). The eggs were incubated at 33°C for 72 h, and the appropriate fluids were harvested after overnight refrigeration.

Serology

The standardized microtiter CF procedure developed at CDC and the standard hemagglutination (HA) and hemagglutination-inhibition (HI) procedures utilizing the proper erythrocyte suspensions were used throughout these studies. Inactivated influenza reagents were evaluated by the neuraminidase inhibition (NI) test. The CDC Inventory of reference antigens and antisera were used as test controls and for evaluating the sensitivity and specificity of all radiation-inactivated reagents.

Experimental procedures

Pooled suspensions of materials infected with each virus were prepared, aliquoted and stored at −70°C. Samples of the pooled suspensions were thawed for radiation experiments. Round, screwcap glass bottles containing approximately 25 ml of virus suspension were placed in the Gammacell sample chamber and mechanically lowered into position for radiation exposure. Throughout exposure, samples were immersed in crushed ice and kept at 4 to 5°C. Inactivation experiments were performed at time intervals corresponding to approximately 0-5 Mrad. Individual samples were removed after radiation treatment ranging from 0-4 to 5-0 Mrad. The samples were held at 4°C and assayed within a few hours for residual live virus. Infectivity assays of untreated and treated suspensions were performed by inoculating viral dilutions (log₁₀ intervals) into the host system used for antigen production. Three screwcap, cell culture tubes or six embryonated eggs were inoculated per dilution. Cultures were incubated at 37°C and examined daily for CPE for 7 to 14 days. Embryonated eggs were incubated at 33°C for 3 days and refrigerated overnight before collection of allantoic and amniotic fluids. For hemagglutinating viruses, HA tests were performed on fluids from individual eggs and culture tubes after three and seven days incubation. Infectivity titers were calculated by the method of Reed & Muench and expressed as either 50% egg infective doses (EID₅₀) or 50% tissue culture infective doses (TCID₅₀).

Confirmation of inactivation was by passage of the fluids from the apparently uninfected cultures at the 10⁻⁰ and 10⁻¹ dilutions of the infectivity titrations. Cell cultures were frozen and thawed before pooling the fluids from each dilution. The pooled passage fluids were inoculated onto the susceptible host and examined for CPE during 7 to 10 days incubation. Inactivation of viruses cultured in embryonated eggs was confirmed by passage of pooled fluids from the 10⁻⁰ and 10⁻¹ dilutions in eggs. After incubation, the fluids from individual eggs were tested for hemagglutinins.

Diagnostic reagents

Antigens were prepared for use in either the complement fixation (CF) or hemagglutination-inhibition (HAI) tests using production procedures described in the Biological Products Procedure Manual. These procedures recommended inactivation using 0-1% BPI. However, instead of BPI, the experimental reagents were inactivated in the Gammacell at a radiation level calculated by the information obtained from the virus inactivation experiments. The volume of diagnostic reagents was 150 to 500 ml. The gamma inactivation procedure was identical to that described
above. After innocuity was confirmed and the antigen potency was determined, the reagents were dispensed in 1 to 5 ml aliquots and stored at either 4°C or -20°C.

Storage stability

Initial experiments involved weekly testing of the influenza reagents. Long-term stability testing for antigen potency and sensitivity was expanded to include CF and HA reagents stored at either 4°C or -20°C over a 9-year period.

Results

Preliminary experiments were conducted to determine the amount of gamma radiation required to inactivate the infectivity of 13 influenza virus strains. Allantoic fluid pools of 400 to 500 ml were prepared for each of the influenza types A and B viruses. An amniotic fluid pool was prepared from eggs infected with influenza C/Taylor/1233/47. The pools were dispensed in 25 ml aliquots in glass, screw-cap bottles and were exposed to five increasing amounts of gamma radiation from 0-4 to 2.1 Mrad. Duplicate infectivity titrations were performed in six embryonated eggs per dilution of the untreated and treated materials. After incubation, the fluids from each egg were harvested individually and tested for hemagglutinins. Positive HA was used as indication of infectivity.

The rates of inactivation were determined by reduction in infectivity titers (Table 2). A/New Jersey/8/76 was inactivated with 0-6 Mrad. This was the smallest radiation dosage required for inactivation of any of the 13 influenza viruses. Four strains were inactivated with 1-3 Mrad and the remaining eight viruses were non-infective after exposure to 1-7 Mrad. Innocuity was confirmed by titration of fluids from individual eggs at the infectivity endpoint dilution. Infectivity titers decreased in direct proportion to the increase in radiation.

As a quality control on the inactivated antigens, 0-1% BPL was used to treat additional samples from each influenza pool by routine procedures. The pools were innocuity tested in embryonated eggs and all were non-infectious after treatment with 0-1% BPL.

The stabilities of untreated versus gamma- and BPL-inactivated hemagglutinins from split samples were compared (Table 3). Gamma and BPL concentrations used were those previously determined to effectively inactivate the influenza antigens. Comparable HA titers were obtained with antigens inactivated by both procedures with the exception of B/Lee/40 which showed a four-fold decrease in titer following BPL treatment. The antigenic specificity was determined in HI tests with reference chicken antisera. Each inactivated antigen was inhibited by reference homologous antisera to a titer within two-fold of that obtained with the non-inactivated control. Radiation with 1-7 Mrad did not cause any change in the antigenic specificity of the antigens. Their performance as reference reagents was unaffected.

Table 2. Determination of the gamma radiation doses required for inactivation at 4°C of 13 different influenza viruses cultured in embryonated chicken eggs

| Viruses               | Untreated | 0-4 | 0-8 | 1-3 | 1-7 | 2-1 |
|-----------------------|-----------|-----|-----|-----|-----|-----|
| A/PR/8/34(H10N1)      | 9.5*      | 3.7 | <1.0| 0   | 0   | 0   |
| A/USSR/90/77(H1N1)    | 8.8       | 5.5 | 2.5 | 0   | 0   | -   |
| A/Brazil/11/78(H1N1)  | 9.0       | 5.3 | 3.0 | 0   | 0   | 0   |
| A/Japan/170/62(H2N2)  | 6.4       | 3.8 | 1.0 | <1.0| 0   | 0   |
| A/Hong Kong/8/68(H2N2)| 8.5       | 5.3 | 2.5 | <1.0| 0   | 0   |
| A/Victoria/3/75(H3N2) | 7.9       | 4.3 | 1.1 | <1.0| 0   | 0   |
| A/Texas/1/77(H3N2)    | 7.8       | 3.9 | 1.0 | <1.0| 0   | 0   |
| A/Equine/Praque/56(Heq1Neq1)| 5.0 | 2.0 | 0   | 0   | 0   |
| A/Equine/Miami/63(Heq2Neq2)| 5.5 | 3.8 | 1.0 | 0   | 0   |
| A/New Jersey/8/76(Hsw1N1)| 7.1 | 4.1 | 0   | 0   | 0   |
| B/Lee/40              | 7.1       | 5.0 | 1.0 | <1.0| 0   | 0   |
| B/Hong Kong/5/72(BX1) | 8.9       | 4.3 | 1.0 | 0   | -   |
| C/Taylor/1233/47      | 7.3       | 5.0 | 1.0 | 0   | 0   | -   |

* Egg/Log10/ID50/ml.
† Specimen not tested.
Table 3. Comparisons of neuraminidase enzyme and hemagglutinin stabilities of influenza viruses after inactivation by either gamma radiation or BPL.

| Viruses                        | Neuraminidase assay* | Hemagglutination† |
|-------------------------------|----------------------|-------------------|
|                               | Gamma (Mrad) 1.7     | BPL 0.1%          | Untreated | Gamma 1.7 | BPL 0.1% |
| A/PR/8/34(H1N1)               | 40                   | 72                | 1280      | 1280       | 1280     |
| A/USSR/90/77(H1N1)            | 22                   | 81                | 320       | 320        | 320      |
| A/Japan/170/62(H2N2)          | 67                   | 58                | 160       | 160        | 160      |
| A/Hong Kong/8/68(H3N2)        | 76                   | 54                | 640       | 640        | 640      |
| A/Victoria/3/75(H3N2)         | 46                   | 55                | 320       | 320        | 320      |
| A/Texas/1/77(H3N2)            | 23                   | 58                | 160       | 160        | 160      |
| A/Equine/Prague/56(Heq1Neq1)  | 80                   | 90                | 320       | 320        | 320      |
| A/Equine/Miami/63(Heq2Neq2)   | 98                   | 93                | 320       | 320        | 320      |
| A/New Jersey/8/76(Hsw1N1)     | 9                    | 91                | 80        | 80         | 80       |
| B/Lee/40                      | 18                   | 44                | 320       | 320        | 320      |
| B/Hong Kong/5/72              | 54                   | 36                | 320       | 320        | 320      |

* Mean of two experiments—Per cent of untreated controls remaining after inactivation by gamma radiation or BPL.
† Titers expressed as reciprocal of highest dilution giving complete hemagglutination.

Using the standard neuraminidase assay, the percentage of neuraminidase activity remaining in the split samples after gamma and BPL inactivation was compared with the percentage of activity in the infectious control preparations. Neuraminidase activity in control and inactivated preparations was tested on at least two occasions (Table 3). Five BPL-inactivated and two gamma-inactivated influenza preparations showed a higher percentage of remaining neuraminidase activity than their corresponding inactivated sample. Four specimens, A/Japan/170/62 (H7N2), A/Victoria/3/75 (H3N2), A/Equine/Prague/56 (Heq1 Neq1), and A/Equine/Miami/63 (Heq2Neq2), exhibited <10% variance between samples in the amount of remaining neuraminidase. These four specimens were considered to be comparable. Greater stability of N1 was observed after BPL inactivation, while Neq1 and Neq2 neuraminidase activity was stable after inactivation by both methods. With the exception of A/Texas/1/77(H3N2), less inactivation of N2 was observed with gamma treatments.

Since greater inactivation of type N1 and B neuraminidases by radiation was observed, additional investigations were performed by repeating the neuraminidase assay on five selected influenza antigens. Influenza A/New Jersey/8/76(Hsw1N1), A/USSR/90/77(H1N1), and B/Hong Kong/5/72 were re-evaluated. A/Texas/1/77(H3N2) was included because of the low result previously obtained and A/equine/Miami/63(Heq2Neq2) was used as a control. These antigens were exposed at five radiation doses, from 0.8 to 4.5 Mrad, under three temperature conditions, unrefrigerated (20°C), ice bath (4°-5°C), and frozen (−20°C) (Fig. 1).

Inactivation of infectivity was slower in the frozen state and required 2.4 Mrad as compared to 1.6 Mrad at other temperatures. The neuraminidase inactivation rates increased with higher temperature and with increased amounts of radiation. At 4°C and above, significant inactivation of the influenza B and N1 neuraminidases was confirmed, but satisfactory levels remained when treatment occurred at −20°C. The N2 neuraminidase level of A/Texas/1/77 showed less inactivation than observed in the previous experiment. However, at temperature above 4°C, significant inactivation occurred at the higher radiation levels that was not evident with the frozen samples. The Neq2 neuraminidase control demonstrated good stability at all temperatures.

The second group of viruses investigated contained 11 enterovirus strains. The objective was to determine the amount of gamma radiation required to inactivate these viruses grown in cell cultures. Enteroviruses 68 and 70 required 3.2 Mrad for inactivation while polioviruses 2 and 3 and coxsackievirus A-4 required 4.2 Mrad. The other enteroviruses were inactivated with 3.7 Mrad (Table 4). Innocuity was confirmed with the pooled suspensions from the endpoint dilutions as previously described. When CPE was not observed after 7 days of incubation at 37°C, the innocuity was considered confirmed.

Six different viruses, three entero- and three myxo-
viruses, were selected from these experiments and the data were plotted on an infectivity destruction rate curve. The rates of gamma inactivation of the different strains tested are similar, and within the limits of the methods used, the dose-inactivation relationship is linear (Fig. 2).

The information obtained in our preliminary experiments was applied to inactivating other viruses and preparing diagnostic reagents. Five different cell cultures served as the host systems for growth of seven of the eight HA antigens and 12 CF antigens. The Newcastle Disease virus HA antigen was grown

Table 4. Inactivation by gamma radiation of 11 enteroviruses grown in cell cultures

| Virus (strain)          | Culture host* | untreated | Gamma Radiation (Mrad) |
|-------------------------|---------------|-----------|------------------------|
| Poliovirus 1 (Mahoney)  | RD            | 9.5†      | 7.5–4.5                |
| Poliovirus 2 (Statler)  | LLC-MK₂       | 8.8       | 6.7–4.7                |
| Poliovirus 3 (Saukett)  | RD            | 8.0–4.5   | 5.5–2.5                |
| Echovirus 9 (Hill)      | LLC-MK₂       | 7.0–4.7   | 3.0–2.5                |
| Coxsackie A-4 (High Point) | RD    | >9.5–6.5  | 3.7–2.5                |
| Coxsackie A-24 (Singapore) | HEL | 7.8–4.3   | 3.3–1.5                |
| Coxsackie B-5 (Faulkner) | LLC-MK₂     | 7.5–4.5   | 2.8–1.5                |
| Enterovirus 68 (Fermon) | HEL          | 7.0–4.3   | 3.3–2.8                |
| Enterovirus 69 (Toluca 1) | HEL | >8.5–6.2  | 3.5–2.2                |
| Enterovirus 70 (J6707/7) | RD          | 8.8–7.3   | 3.7–1.5                |
| Enterovirus 71 (BrCr)   | RD            | 9.3–5.8   | 3.7–1.5                |

* See Table 1 for explanation of culture host.
† Log₁₀(TCID₅₀)/ml.
‡ Specimen not tested.
in 10-day embryonated eggs. Infectivity titers were determined for each pool and the antigens were gamma-inactivated while chilled in an ice bath at 4 to 5°C. The serologic reactivity was compared with that obtained with non-inactivated materials (Table 5). Innocuity tests were performed and confirmed in the same host system. The radiation dosage necessary for inactivation varied with each of the viruses.

During inactivation, HA titers for measles, Newcastle Disease, and parainfluenza types 1 and 3 remained stable. Reovirus types 1 and 2 showed a four- to eight-fold decrease in titer, but retained satisfactory potency. Reovirus type 3 HA antigen exhibited an eight-fold titer decline to an unsatisfactory potency. The HA titer of simian rotavirus was totally destroyed. In comparing the CF titers of non-irradiated and irradiated antigens, we observed that two antigens, coronavirus and respiratory syncytial showed a >two-fold decrease in potency after gamma treatment. Three antigen preparations (parainfluenza 1, calf and simian rotaviruses) were unsatisfactory because of low potency levels in both treated and nontreated preparations. The remaining CF antigens were satisfactory. The sensitivity and specificity of the inactivated HA and CF antigens were comparable to that of the CDC reference antigens as

| Table 5. Complement fixation and hemagglutination titers of 16 different viral antigens comparing pre-tested titers with those obtained after gamma inactivation |
|---------------------------------------------------------------|
| Viruses            | Host       | Infection titers* | Gamma inactivation† | CF titers       | HA titers       |
|--------------------|------------|-------------------|---------------------|-----------------|-----------------|
|                    |            |                   |                     | Non-rad         | Rad             |
| Coronavirus        | HEL        | 6-5               | 0-98                | 8;              | 2               |
| Cytomegalovirus    | HEL        | 2-5               | 0-42                | 8               | 4               |
| Herpesvirus 1      | VERO       | 9-0               | 1-01                | 64              | 128             |
| Herpesvirus 2      | VERO       | 5-5               | 0-98                | 8               | 8               |
| Measles            | HEL        | 5-0               | 0-80                | 256             | 256             |
| Newcastle Disease  | Egg/Alf    | 7-6               | 1-98                | —               | 160             |
| Parainfluenza 1    | PrRhK      | 5-2               | 0-43                | 2               | 2               |
| Parainfluenza 3    | VERO       | 6-0               | 1-18                | 8               | 8               |
| Poliovirus 1       | RD         | 9-0               | 3-70                | 32              | 16              |
| Resp. Syncytial    | VERO       | 4-5               | 1-73                | 8               | 2               |
| Reovirus 1         | LLC-MK₂     | 6-2               | 1-73                | —               | 512             |
| Reovirus 2         | LLC-MK₂     | 7-3               | 2-15                | —               | 4096            |
| Reovirus 3         | LLC-MK₂     | 4-2               | 1-28                | —               | 128             |
| Rotavirus, calf    | MA-104     | 6-8               | 1-15                | 2               | 2               |
| Rotavirus, simian  | MA-104     | 6-5               | 2-28                | 2               | 256             |
| Varicella          | HEL        | 4-5               | 0-84                | 16              | 8               |

* Log_{10}/TICID_{50}/ml NDV Log_{10}/RID_{50}/ml.
† Gamma radiation inactivating dosage in Mrad.
‡ CF titers expressed as reciprocal of endpoint antigen dilution in block titration.
§ HA titers expressed as reciprocal of highest dilution giving complete hemagglutination.
Table 6. Stability of 28 lots of gamma-inactivated hemagglutinating antigens for 13 different influenza viruses evaluated during 9 years of storage at 4°C

| Virus Lot no. | 0 | 1 | 3 | 5 | 7 | 9 |
|--------------|---|---|---|---|---|---|
| A/Japan/305/57 90-0134 | 320* | 320 | 320 | — | — | 160 |
| A/Texas/1/77 (IVR6) 80-0160 | 1280 | 1280 | — | — | — | 1280 |
| A/Texas/1/77 83-0085 | 320 | 160 | — | 80 | — | — |
| A/Bangkok/1/79 80-0062 | 320 | 320 | — | 320 | — | 160 |
| A/England/333/80 80-0063 | 640 | 640 | 320 | — | — | 320 |
| A/Philippines/2/82 81-0025 | 640 | — | — | 160 | — | — |
| A/Chile/1/83 81-0065 | 320 | — | — | 80 | — | — |
| B/Lee/40 82-0038 | 320 | — | 320 | — | 160 | — |
| B/Massachusetts/1/71 81-0105 | 640 | 640 | 640 | — | 160 | — |
| B/Hong Kong/5/72 83-0032 | 160 | — | — | 80 | — | — |
| B/Singapore/222/79 83-0033 | 160 | 160 | — | 80 | — | — |
| B/SSSR/100/83 84-0060 | 320 | — | — | 80 | — | — |
| C/Taylor/1233/47 85-0136 | 320 | — | 320 | — | — | — |
| 86-0107 | 320 | — | 160 | — | — | — |
| B/USSR/100/83 81-0105 | 160 | 320 | 320 | — | 160 | — |
| B/USSR/100/83 80-0038 | 1280 | 1280 | — | — | 320 | — |
| B/USSR/100/83 80-0041 | 320 | — | 320 | — | 320 | — |
| B/USSR/100/83 81-0056 | 640 | 160 | 160 | — | 160 | — |
| B/USSR/100/83 82-0096 | 640 | — | — | 80 | — | — |
| B/USSR/100/83 82-0096 | 640 | — | — | 80 | — | — |
| C/Taylor/1233/47 79-0054 | 320 | — | 160 | — | — | 40 |
| C/Taylor/1233/47 82-0146 | 160 | 160 | — | — | 160 | — |

* HA titers expressed as reciprocal of highest dilution giving complete hemagglutination. Minimum acceptable titer 160.
† Specimen not tested.

determined in tests with homologous and heterologous the CDC reference human and animal antisera. Interference from anticomplementary activity was not observed with the CF antigens.

The stability of stored diagnostic reagents has always been a concern of reagent manufacturers. We examined six HA and seven CF gamma-inactivated antigens held under routine storage conditions for approximately 5 years. The HA antigens for measles, Newcastle Disease, parainfluenza types 1 and 3, and reovirus types 1 and 2 were evaluated. All antigens maintained stability within ±two-fold titer variations. Long-term stability of cytomegalovirus, herpes types 1 and 2, measles, parainfluenza 3, poliovirus 1 and varicella CF antigens were evaluated. Six of the seven CF antigens maintained titer stability through the 5-year period. The herpesvirus type 1 CF antigen showed a four-fold decrease in titer but still maintained an acceptable potency level.

Thirteen influenza viruses, represented by 28 HA antigen preparations, were examined periodically after liquid storage at 4°C for periods up to 9 years. Using 1:160 as the minimum acceptable influenza HA antigen titer, satisfactory results were observed with all antigens during 3 years storage and with 71% of the antigens over 5 years storage (Table 6). Of the 16 antigens available for testing after 7 years storage, 88% gave satisfactory HA titers and 86% of the antigens stored for 9 years demonstrated satisfactory HA titers.
Discussion

This study sought to ascertain the effect of gamma radiation on the potency and sensitivity of viral CF and HA antigens. The findings were applied to the production of high quality diagnostic reagents. Initial experiments involved allantoic fluid cultures infected with 13 different strains of influenza virus. Gamma inactivation procedures were compared to the standard technique using BPL. The antigens were evaluated by HA, HI and neuraminidase assay. The temperature during radiation had a definite effect on the stability of the influenza virus neuraminidase. The HA potency of the gamma-inactivated antigens was comparable to that obtained with the BPL-inactivated antigens.

After determining that gamma-inactivated influenza HA antigens retained potency and specificity, we evaluated this technique on the preparation of CF and/or HA antigens for other viruses. These viruses were inactivated by gamma radiation of 0.42 to 3.70 Mrad without adverse effect on the potency, sensitivity or specificity of the antigen. Our results agree with other reports\textsuperscript{12,17} that the efficacy of inactivation of viral infectivity by gamma radiation depends on the optimal radiation exposure. The radiation required for inactivation varies among virus groups. As reported by Thomas \textit{et al.}\textsuperscript{2} inactivation occurs along predictable linearity of the lethal dose levels.

From the kinetics of the virus inactivation, it is possible to calculate the amount of radiation required to destroy the infectivity and still retain the antigenicity of the reagent. If a given radiation dosage produces incomplete inactivation, the suspension can be subjected to a further treatment which may be calculated from the standard inactivation curve.

In general, inactivated virus suspensions, either cell culture or allantoic fluids, gave results similar to noninactivated preparations in the HAI and CF tests. Exceptions were noted with reovirus 3 and simian rotavirus HA antigens which after inactivation decreased to unacceptable levels. A four-fold drop was observed with the respiratory syncytial and coronavirus CF antigens. Inactivation did not decrease the potency or specificity of three antigen suspensions which had a 1:2 CF titer. These antigens were already considered below acceptable titers.

For a number of years the CDC viral CF and HA antigens have been inactivated with gamma radiation. An evaluation of the stability of influenza HA antigens after long-term storage is reported (Table 6). Results are included for antigens of several influenza strains not discussed in the preliminary experiments. The results presented show a high percentage of antigens maintaining satisfactory potency of $\geq 160$ after long term storage.

Laboratory workers are at considerable risk of infection from handling large volumes of infectious viral suspensions used in preparing diagnostic reagents. They also face exposure to hazardous chemicals, including BPL, used in the processing procedures. These results indicate that gamma radiation effectively and reliably inactivates high-titered virus suspensions without affecting the serologic specificity. This procedure is safer than chemical inactivation and is an effective replacement for BPL in the preparation of inactivated diagnostic reagents.

Acknowledgement

We thank Alan P. Kendal for his advice regarding neuraminidase inactivation; Judith C. Galphin for technical assistance with the neuraminidase assays; Martha B. Fears and Avis G. Daugharty for technical assistance in the serologic evaluations; and W. Adrian Chappell for his helpful comments and support throughout the project.

References

1. Elliott LH, McCormick JB, Johnson KM. Inactivation of Lassa, Marburg, and Ebola viruses by gamma irradiation. J Clin Microbiol 1982; 16: 704–708.
2. Thomas FC, Davies AG, Dulac GC, Willis NG, Papp-Vid G, Girard A. Gamma ray inactivation of some animal viruses. Can J Comp Med 1981; 45: 397–399.
3. Sullivan R, Fassolitis AC, Larkin EP, Read RB Jr, Peeler, JT Inactivation of thirty viruses by gamma radiation. Appl Microbiol 1971; 22: 61–65.
4. White LA, Chappell WA. Inactivation of enteroviruses by gamma radiation. Abstr Annu Meet Am Soc Microbiol 1983; 305.
5. White LA, Hall HE, Chappell WA, Kendall AP. Inactivation of influenza virus antigens by gamma radiation. Abstr Annu Meet Am Soc Microbiol 1979; 272.
6. White LA, Freeman CY, Chappell WA. Inactivation of viral antigens by gamma radiation. Abstr Annu Meet Am Soc Microbiol 1980; 261.
7. Tzianabos T, Campbell RC, Wright GG. Preparation of noninfectious arbovirus antigens by sucrose-acetone extraction of gamma-irradiated tissue. Proc Soc Exp Biol Med 1971; 136: 187–190.
8. Polley JR. Preparation of stable noninfective influenza virus antigens for typing by hemagglutination-inhibition. Can J Microbiol 1969; 15: 203–207.
9. Sullivan R, Scarpino PV, Fassolitis AC, Larkin EP, Peeler JT. Gamma radiation inactivation of coxsackievirus B-2. Appl Microbiol 1973; 26: 14–17.
10. Thomas FC, Ouwerkerk T. McKercher P. Inactivation by gamma irradiation of animal viruses in simulated laboratory effluent. Appl Environ Microbiol 1982; 43: 1051–1056.
11. Chappell WA, White LA, Gamble, WC. Production manual for viral rickettsial chlamydial mycoplasmal reagents, 6th edn, Atlanta, GA, Centers for Disease Control, 1984.
12. Joklik WK. Inactivated viral vaccines. In: Joklik WK, ed. Principles of Animal Virology. New York: Appleton-Century-Crofts, 1980: 156.
13. Palmer DF, Whaley SD. Complement fixation test. In: Rose NR, Friedman H, Fahey JL, eds. Manual of Clinical Laboratory Immunology, 3rd edn., Washington, DC: American Society for Microbiology, 1986: 57–66.
14. Hierholzer JC, Suggs MT, Hall EC. Standardized viral hemagglutination and hemagglutination-inhibition tests. II. Description and statistical evaluation. Appl Microbiol 1969; 18: 824–833.
15. Kendal AP, Pereira MS, Skehel JJ. Concepts and procedures for laboratory-based influenza surveillance. Atlanta, GA, Centers for Disease Control, 1982.
16. Reed LJ, Muench H. A simple method of estimating fifth per cent endpoints. Am J Hyg 1938; 27: 493–497.
17. Polley JR. The use of gamma radiation for the preparation of virus vaccines. Can J Microbiol 1962; 8: 455–459.

Received for publication 2 January 1990; accepted 20 May 1990.