Chapter 10
Preclinical Imaging in BSL-3 and BSL-4 Environments: Imaging Pathophysiology of Highly Pathogenic Infectious Diseases

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Abstract Structural and functional imaging are emerging as powerful tools for studying highly pathogenic infectious disease processes. Nuclear imaging modalities and sophisticated radiolabeled probes can be used to track physiological or biochemical processes associated with viral infection. Magnetic resonance imaging can provide anatomical images with exquisite soft tissue contrast, while magnetic resonance spectroscopy can measure the relative amounts of certain metabolites in a given tissue. However, conducting medical imaging studies in a high-containment laboratory requires advanced applications and modification not only of image acquisition and analysis processes but also of the imaging equipment. Processes such as ex vivo labeling of cells are hampered by the personal protective equipment required for the safety of laboratory personnel. Modification of medical imaging equipment can prevent contamination of the equipment. Regardless of the challenges involved, medical imaging could provide valuable information to researchers developing therapeutics against highly pathogenic infectious diseases.

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10.1 Benefits of Medical Imaging in Infectious Disease Research and Drug Development

In vivo medical imaging is emerging as a powerful supplement to conventional studies of viral pathogenesis and treatment. Imaging techniques have the potential to become influential and informative tools that could be applied to many steps in the drug development process. In general, one substantial benefit associated with medical imaging in highly pathogenic infectious disease research and drug development is the potential for longitudinal studies in a single subject with minimal interference with physiological processes and disease development. As opposed to conventional studies based around euthanasia and necropsy of infected subjects at various time points along the disease process, this longitudinal approach could increase the statistical relevance while decreasing the number of subjects needed to assess the efficacy of an investigational drug or treatment (Rudin and Weissleder 2003).

The drug development process is a time-consuming and expensive venture that involves many steps prior to approval. Both the financial and temporal burdens of many of the required phases might be mitigated with the use of medical imaging technologies. For example, the first step in the development of a new drug or biologic is the identification of the drug target, such as proteins and enzymes encoded by viruses. By developing and using specialized probes that bind to the drug targets, medical imaging could verify the presence and characterize the spatial and temporal distribution of intended targets (Willmann et al. 2008). After a target is chosen, high-throughput screening of compound libraries identifies experimental drugs or chemicals that have the desired effect on the target (Willmann et al. 2008; Valadon et al. 2006). Molecular imaging techniques could assess gene–protein interactions during this step of compound identification (Luker et al. 2003; Sharma et al. 2002). Also, by labeling investigational drugs, imaging could characterize the pharmacokinetics (e.g., accumulation and distribution over time) using various routes of administration and dosing schedules (Viglianti et al. 2004; Garg et al. 2008; Di Mascio et al. 2009; Bray et al. 2010; Ferro-Flores et al. 2012). The biodistribution of a drug interpreted from an image might give the first indication of the drug interacting with the intended target (Valadon et al. 2006). For example, small interfering RNAs (siRNAs) used to produce a therapeutic effect could be radiolabeled or conjugated to magnetic nanoparticles to verify tissue distribution or delivery of the molecules to the intended target using nuclear or magnetic resonance imaging (MRI) (Liu et al. 2007; Kumar et al. 2010). Furthermore, imaging techniques have the potential to quantify viral burden, assess the severity of infection or inflammation, track the host response to disease processes, and evaluate treatment outcomes throughout the life of the subject (Bray et al. 2010; Schellingerhout et al. 1998; Nahrendorf et al. 2006; Dotti et al. 2009; Jubeli et al. 2012). Biological, physiological, and molecular parameters assessed or quantified by imaging modalities may aid the drug development process by predicting the efficacy or safety of a new drug and serving as a surrogate for a clinical endpoint in controlled studies (Willmann et al. 2008; Wang and Deng 2010). Development of these parameters, collectively referred to as imaging biomarkers, could also facilitate evaluations of novel drug therapy (Rudin and Weissleder 2003).

Other considerations for the development of drugs and therapies for high consequence pathogens include the implementation of the FDA's "Animal Rule" (US Food and Drug Administration 2002). This rule applies to new drugs or biologics that are intended to treat or prevent disease that are either (1) very rare or (2) life-threatening or permanently debilitating. In the first case, clinical studies involving large patient populations are impractical, and historical data on these diseases are limited. In the second case, human studies are unethical, for obvious reasons. In these situations, investigators may forgo clinical trials if efficacy of a new drug or treatment procedure is evidenced by adequate well-controlled animal studies. Medical imaging could be used to support the assertion that the pathogenesis of the disease under question in selected animal models is similar to that in humans.

10.2 Image Acquisition and Analysis Strategies for Infectious Disease Research

10.2.1 Nuclear Imaging

Nuclear imaging modalities such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are powerful, noninvasive techniques to visualize both location and number of infectious and inflammatory foci throughout the body. These techniques track physiological or biochemical processes at cellular and molecular levels. Application of nuclear imaging modalities focusing specifically on viral infection and diseases associated with biosafety level-3 (BSL-3) or BSL-4 pathogens has been limited. However, molecular imaging could provide a better understanding of viral infection progression and specific physiologic and metabolic changes that correspond to the efficacy of pharmaceutical therapies.

Clinically, a host response to infection can be studied by use of available isotopes and radiotracers that are not specific for a viral infection. Common features of most immune responses to an infection include increased blood supply, local or systemic changes in vascular permeability, and enhanced transudation. Beginning in the 1970s, cytomegalovirus pneumonitis and other infections in immunodeficient patients could be detected by administering an intravenous dose of Ga-67, which binds to circulating transferrin, and the in vivo biodistribution can be subsequently imaged with a gamma camera (Hamed et al. 1979; Reinders Folmer et al. 1986). Similar to other radiolabeled macromolecules such as proteins, polysaccharides, and polyamine derivatives proposed for imaging inflammation, Ga-67-transferrin complex extravasates at the site of inflammation as a result of increased blood flow and enhanced vascular permeability.

Other approaches to imaging infection include the intravenous infusion of In-111- or Tc-99m-labeled polyclonal human immunoglobulin (IgG), which is retained at sites of inflammation due to specific binding of IgG with Fc receptors

expressed on leukoctyes, which accumulate at the site of infection (Rubin and Fischman 1994). Radiolabeled IgG accumulation at infectious foci is mainly attributable to nonspecific extravasation or leakage of the labeled protein associated with increased vascular permeability. IgG radiolabeled with In-111 appears to have both high sensitivity and specificity for imaging human immunodeficiency virus (HIV)-infected patients (Buscombe et al. 1995).

A common practice in nuclear imaging is to use radiolabeled leukocytes that migrate to sites of infection and localize the sites of inflammation (Kumar 2005). Two approaches can be used for leukocyte labeling: in vivo and ex vivo. In vivo leukocyte labeling can be based on either antibody–antigen interaction or leukocyte receptor binding. Ex vivo labeling requires drawing a blood sample from the subject, isolating and purifying leukocyte population, radiolabeling with the appropriate radionuclides such as Tc-99m or In-111, and reinjecting the leukocytes into the subject. Ex vivo labeling requires additional lab space and equipment, is time intensive, and can subject laboratory staff to increased risk if blood samples contain a pathogenic agent. Therefore, ex vivo leukocyte labeling is not optimal for a BSL-4 environment. Regardless of the labeling strategy, imaging with radiolabeled leukocytes provides up to 90 % sensitivity for detection of both acute and chronic infection in the clinical setting (Wanahita et al. 2007).

Another way to image sites of viral infection or inflammation is with F-18 fluorodeoxyglucose (F-18 FDG), a nonspecific PET radiotracer that is trapped in cells in proportion to glycolytic activity. F-18 FDG PET imaging can be used to develop and evaluate new drugs for infectious diseases and monitor immune responses to newly developed treatments. Early in the HIV pandemic, for example, PET imaging with F-18 FDG demonstrated that regional or generalized alterations of cerebral glucose metabolism accompanying functional brain impairment in acquired immunodeficiency syndrome (AIDS) dementia can be reversed after effective antiviral (zidovudine) therapy (Yarchoan et al. 1987; Brunetti et al. 1989). The same technique has shown that peripheral lymph nodes have a greater glucose uptake in untreated HIV-infected patients than in patients on antiretroviral therapy, indicating that lymph nodes are a site of viral replication (Brust et al. 2006) or, alternatively, the increase could be due to uptake of F-18 FDG by clonal expanding lymphocytes. Computed tomography (CT) and F-18 FDG PET imaging of a patient with severe swine-origin H1N1 influenza indicated an intense inflammatory response revealed by an increase in F-18 FDG uptake in areas of dense pulmonary consolidation (characterized by higher Hounsfield units on CT images) as well as in regions of aerated lung (Bellani et al. 2010).

However, F-18 FDG is a nonspecific probe and incapable of differentiating inflammation from an infectious process. Therefore, other radiotracers that specifically quantify or characterize cell proliferation have been developed. For example, F-18 fluoro-3-deoxy-3-L-fluorothymidine (F-18 FLT) is monophosphorylated by thymidine kinase 1 (TK1) and remains trapped inside cells (Bading and Shields 2008). Since TK1 concentrations increase during the synthesis phase of the cell cycle, the uptake of F-18 FLT is believed to correlate with cell proliferation rather than with metabolic activity. Thus, F-18 FLT might be useful for identifying more

specific processes and mechanisms associated with immune response. Nevertheless, neither F-18 FDG nor F-18 FLT has any specificity towards any particular cell or tissue type. In contrast, 1-(29-deoxy-29-18F-fluoro-b-L-arabinofuranosyl)cytosine (F-18 FAC) represents a specific probe for lymphoid tissue (Bray et al. 2010; Shu et al. 2010). This probe targets deoxycytidine kinase, an enzyme involved in DNA synthesis through deoxyribonucleoside salvage pathway. Since deoxycytidine kinase is primarily expressed in lymphoid cells, F-18 FAC is considered a specific probe to study immune activation in vivo.

A different application of molecular imaging in infectious disease research is to label viruses directly using specific probes that bind to or are incorporated into viruses. The value of virus-specific radiopharmaceuticals is not only in the development of therapeutics against dangerous pathogens in animal models but also is to diagnose and formulate clinical decisions regarding therapy. New and improved radiotracers are being developed from several efficient antiviral drugs known to inhibit viral replication by binding to specific virion structural proteins or to the active sites of a viral enzyme. In addition, some antibodies are active against viral proteins expressed on the surface of infected cells. Such drugs and antibodies have been proposed for use as radiolabeled probes for the detection of viral infection (Bray et al. 2010) to visualize sites of viral replication in the body by in vivo imaging. Bray et al. identified a number of processes unique to viral replication that might serve as targets for radiolabeled, pathogen-specific tracers. They reviewed nine different DNA and RNA virus families and identified approved and experimental antiviral drugs that target virus-encoded molecules that might have potential as radiolabeled probes. A current example of this approach is the PET imaging of herpes simplex virus infections, in which the viral thymidine kinase phosphorylates thymidine analogues labeled with several different radionuclides, including C-11 or I-131, trapping them within infected cells (Gambhir et al. 2000). Finally, virus particles can be radiolabeled and tracked by in vivo imaging, providing a quantitative measure of viral burden (Schellingerhout et al. 1998; Rojas and Thorne 2012; Penheiter et al. 2012).

10.2.2 Magnetic Resonance Imaging

In contrast to other imaging modalities, MRI offers high, potentially submillimeter spatial resolution. Also, MR images exhibit superior soft tissue contrast compared to other imaging modalities, since the magnetic characteristics of tissues with similar electron densities can differ considerably. Magnetic resonance spectroscopy (MRS) measures the presence and relative amounts of certain metabolites based on the resonant frequencies of the metabolites present within a sample. Furthermore, unlike nuclear imaging, MR images can be obtained by exploiting only endogenous sources of contrast; exogenous and potentially harmful contrast agents are not a necessity. However, as will be discussed below, contrast agents can be used to probe certain cellular or molecular interactions. In general, an appreciable amount of flexibility is

associated with magnetic resonance techniques. This flexibility makes the modality appealing for infectious disease research in BSL-3 or BSL-4 environments.

Many viral hemorrhagic fevers (VHFs) are caused by pathogens that must be contained within a BSL-3 or BSL-4 environment. As the name suggests, VHFs are characterized in part by overall damage to the vascular system, often accompanied by hemorrhage and an increase in vascular permeability. A number of MRI techniques assess vascular permeability, integrity of the blood–brain barrier, and prevalence of hemorrhages in other nonviral diseases. Previously, dynamic contrast-enhanced MRI has been using to assay and quantify vascular permeability (Padhani et al. 2000; Floris et al. 2004; O'Connor et al. 2007; Cyran et al. 2012). Using an FDA-approved gadolinium diethylenetriaminepentaacetate (Gd-DTPA) contrast agent, Floris et al. reported a statistically significant increase in T1 values on quantitative T1 maps that were hypothesized to result from increased vascular permeability and subsequent leakage of the contrast agent. These measurements were used to objectively measure blood–brain barrier integrity (Floris et al. 2004). Cyran et al. used a macromolecular contrast medium and a dynamic image acquisition to estimate vascular permeability of tumors using a two-compartment model (Cyran et al. 2012). The calculated coefficients for endothelial permeability correlated with immunohistochemical measurements of vascular endothelial growth factor. Also, susceptibility-weighted imaging (Haacke et al. 2004) has been shown to be more sensitive in detecting hemorrhages and differentiating them from other physiological processes than classic T2*-weighted imaging techniques (Lobel et al. 2010). Liu et al. have used quantitative susceptibility mapping to objectively measure and assess the presence of as well as the physiologic burden of cerebral microhemorrhage (Liu et al. 2012). Approaches similar to these techniques would be beneficial to tracking both the loss of vascular integrity and prevalence and burden of hemorrhage with the progression of VHFs.

In addition to quantifying vascular permeability, MRI could potentially be used to track cell migration during viral infections. Recently, strategies for imaging cellular migration with MRI have been applied to tracking the distribution of stem cell therapies (Hoehn et al. 2007). In order to achieve a detectable level of contrast, cells of interest must be labeled prior to imaging. Many different, well-documented in vitro labeling strategies exist (Frank et al. 2003; Modo et al. 2005). However, similar to PET imaging tracers described above, in vivo labeling is preferred in a BSL-4 environment due to increased simplicity of the labeling process and avoidance of the risks associated with handling infected cells or tissue. Spontaneous, in vivo labeling of monocytes, macrophages, and other phagocytic cells can be achieved by systemic injection of a contrast agent that subsequently is incorporated into the cell by phagocytosis. Labeled cells then infiltrate areas of inflammation. Iron oxide nanoparticles, specifically ultrasmall paramagnetic iron oxides (USPIOs) or superparamagnetic iron oxides (SPIOs), have been successfully used as contrast agents to label phagocytic cells. Iron oxides produce areas of signal void, or hypointensity, in T2- or T2*-weighted MR images. In vivo labeling has been used to track macrophage infiltration in rat models of antigen-induced arthritis (Beckmann et al. 2003), inflammatory neurological disorders (Stoll et al. 2004), and ischemic brain

lesions (Kleinschnitz et al. 2003). Macrophage tracking would be extremely beneficial in the investigation of many infectious diseases. However, one challenge associated with this technique is the difficulty in distinguishing between SPIO-labeled cells and endogenous blood derivatives associated with hemorrhage that also exhibit hypointensity (Bulte 2009). This difficulty with detection of SPIO-induced loss of signal intensity may limit the efficacy of using SPIO-labeled cells for imaging viral hemorrhagic fevers or other diseases caused by BSL-3 or BSL-4 pathogens that are associated with hemorrhage.

Although viral hemorrhagic fevers or diseases caused by other exotic, high-consequence (BSL-4) viruses have not been imaged with MRI, patients infected with certain neurotropic viruses (BSL-3 viruses) have been imaged using MRI in the clinical setting. Japanese encephalitis virus is endemic in Southeast Asia (Umenai et al. 1985). Bilateral hemorrhagic thalamic involvement (Kumar et al. 1997) and bilateral white matter lesions (Shoji et al. 1994) have been detected by MRI in such virus-infected patients. Large outbreaks of West Nile virus disease occurred in United States in 2002 and 2003 (Hayes and Gubler 2006). Hyperintense lesions in the leptomeninges, cortex, subcortical white matter, brainstem, thalamus, and substantia nigra can be observed on T2-weighted clinical images (Jeha et al. 2003; Sejvar et al. 2003; Burton et al. 2004). While these publications are an appreciable benefit to clinical practice, well-controlled, experimental MRI studies in high-containment laboratories have not characterized the radiological presentation of these diseases. Such studies would be advantageous to both clinical diagnosis and drug treatment development.

10.2.3 Image Analysis

Many robust medical image processing techniques have been developed and validated in human subjects and phantoms in clinical and preclinical settings. To integrate medical imaging and infectious disease research in a BSL-3 or BSL-4 laboratory, techniques optimized for humans outside of containment need to be translated to small animals in high-containment environments. This translation is not without challenges.

For longitudinal studies of disease progression, all temporal scans of the subject should be aligned to the same spatial coordinate system using registration techniques. Such alignment is critical in providing quantitative data of rate of change of features during disease progression. This alignment is normally performed by automated image registration methods that benefit from considerable care in positioning the subject correctly and reproducibly in the scanner. In BSL-3 and BSL-4 environments, optimal subject placement is inherently more difficult due to restrictions of movement of personnel wearing positive-pressure suits or other personal protective equipment. Therefore, image registration methods must be robust to larger positional differences in subject orientation across scanning sessions in high containment than that occurring during sessions outside containment.

Atlas-based segmentation has become a common method to delineate anatomy in a medical image. For example, atlases of liver, brain, and other organs are used for image overlays in human research studies. In BSL-3 and BSL-4 environments, new animal atlases must be developed to utilize these segmentation strategies. Image segmentation is also more difficult in small animals than in humans due to the significantly smaller anatomy and corresponding decrease in the number of volume elements, or voxels, which represent a given area of interest within the image.

Many of the medical image analysis methods that have been developed and extensively validated were designed for the initial purpose of neuroimaging research (e.g., traumatic brain injury). These neuroimaging analysis methods must be translated to other organ systems necessary for the characterization of infectious disease and development of treatment.

10.2.4 Discovery and Validation of Imaging Biomarkers for Infectious Disease

Increasingly, medical imaging modalities provide either imaging biomarkers or surrogate endpoints for the costly and time-consuming process of drug development (Pien et al. 2005). An imaging biomarker is defined by extension of the Biomarkers Definitions Working Group (Biomarkers Definitions Working Group 2001) as "any anatomical, physiological, biochemical or molecular parameter detectable by one or more imaging modalities used to establish the presence or severity of disease" (Richter 2006). For example, the number of lesions identified on a T2-weighted MR image has been used as a surrogate endpoint for evaluating treatment response in patients with multiple sclerosis (Smith et al. 2003). Dynamic contrast-enhanced MRI markers, including blood flow, vessel permeability, or blood volume, have been used to assess reduction of vascularization of tumors in studies of antiangiogenic drugs (O'Connor et al. 2007). Enhanced standardized uptake value determined from an F-18 FDG PET image has been used as an imaging biomarker for tumor metabolism (O'Connor et al. 2008).

Traditionally, investigations into infectious disease pathogenesis or the development of vaccinations or therapeutics against these diseases rely heavily upon clinical assessments, clinical assays, standard techniques of virology and immunology, and histopathological findings in animal models. An appreciable challenge to integrating medical imaging into research conducted in high containment is the determination, development, and validation of imaging biomarkers that will quantify disease processes associated with the pathogens. Imaging studies performed with the intent of biomarker development must be prudently designed to correlate imaging biomarker data with at least one other, well-established method to prove the accuracy, precision, and sensitivity of the imaging biomarker (Smith et al. 2003). The risks of relying upon imaging biomarkers that have not been sufficiently validated beyond an individual study can be highly prejudicial, as these unvalidated biomarkers could negatively impact future studies.

Current and potential applications of clinical imaging modalities to infectious disease research have been reviewed above; however, the applications discussed were not necessarily designed to fulfill the requirements of an imaging biomarker. In an experimental study of nonhuman primate monkeypox virus (MPXV) disease model of human variola infection, serial F-18 FDG PET imaging has been used to identify inflammatory patterns of MPXV infection as predictors of disease outcome (Dyall et al. 2011, 2012). Multiple processes can contribute to lymph node activation. Such processes include, but are not limited to, immune cell proliferation as a part of a normal immune response, inflammation from cytocidal effects of MPXV, or cytotoxicity associated with the infiltration of activated immune cells.

Various imaging modalities have been used to identify biomarkers in animal models of infectious diseases. For example, magnetic resonance spectroscopy was used to identify changes in metabolic markers of neuronal integrity, such as N-acetylaspartate or creatine, during minocycline therapy of simian immunodeficiency virus-induced encephalitis (Ratai et al. 2010). In another study, the disease pathogenesis of neuroAIDS in nonhuman primates was monitored using C-11 (R)-1-(2-chlorphenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline-carboxamide, a PET radioligand for peripheral benzodiazepine receptors abundant on macrophages. Increased binding of this radioligand has been proposed as a biomarker of neurodegenerative disease (Venneti et al. 2004).

10.3 Containment Strategies to Incorporate Imaging Platforms

The integration of modern medical imaging technologies within a high-containment biological laboratory is a relatively new concept that has not been widely undertaken. This lack of integration is mainly due to concerns over breaching the biocontainment barrier, contaminating the imaging equipment and potentially harming the imaging equipment with harsh decontamination chemicals. Furthermore, many of the pathogens for which vaccines or therapies are actively sought pose significant health risks to humans and therefore call for high levels of biosafety in highly specialized laboratory spaces. Such laboratory spaces require specialized ventilation and air treatment systems, controlled-access zones, air locks at laboratory entrances, and separate building modules to isolate the laboratory (US Department of Health and Human Services, Centers for Disease Control and Prevention, National Institutes of Health 2009).

Several containment strategies integrate medical imaging into the theatre of infectious disease research within a BSL-3 or BSL-4 laboratory. The majority of these strategies fall under one of two categories: imaging equipment that is fully contained within BSL environment or the BSL environment is extended to include only necessary components of the imaging equipment. Some advantages and difficulties inherent to each strategy are discussed below.

10.3.1 Fully Contained Equipment

One method for the integration of medical imaging and biological hazards is the inclusion of the entire imaging instrument within a high-containment laboratory. This method differs from the others discussed in this section in that there is no physical biocontainment barrier between imaging equipment and infected subject. As such, full containment of imaging instruments is only practical in circumstances when the instrument in question is dedicated to infectious disease imaging. Such containment would not only limit the use of the imaging device but would also be associated with a relatively large footprint within the BSL laboratory. A major concern in employing this configuration is the ability to decontaminate and service the devices without voiding warranties.

This containment configuration would require service engineers to have access to the high-containment laboratory to perform maintenance or service tasks. Such access could be granted in one of two scenarios. First, the service engineers are trained in BSL-3 or BSL-4 laboratory practices, and they perform required tasks using these practices, including donning personal protective equipment. The entry and training requirements for work within a BSL-3 or BSL-4 laboratory are not trivial, especially when certain high consequence pathogens are involved (US Department of Agriculture 2005). Requiring this training for each service engineer would be time consuming and inefficient. Furthermore, maintenance or repair activities could require work with tools or objects that may increase the risk of a tear in the positive-pressure suit or other breaches of personal protective equipment.

Alternatively, the imaging suite could be decontaminated prior to planned maintenance or repair by service engineers who are not trained in BSL practices. Subjecting the imaging device to decontamination procedures may increase the risk of damage. Many chemicals used in decontamination of a BSL-3 or BSL-4 laboratory (US Department of Health and Human Services, Centers for Disease Control and Prevention, National Institutes of Health 2009) could harm the device. Caustic etching of detectors, progressive degradation of device wiring insulation, and corrosion of solder points are only a few of the risks. Therefore, neither of these scenarios (i.e., entry of trained service engineers, decontamination) is optimal.

10.3.2 Containment Extension Strategy

Containment extension strategies are those that aim to keep the imaging device itself, or a large portion of it, outside the high-containment zone. Such strategies are solutions for imaging modalities that are large and not easily integrated into the BSL-3 or BSL-4 laboratory for the reasons described above, for example, clinical CT, PET, SPECT, and MRI systems. Containment is maintained by creating an extension of the high-containment laboratory within the bore or surrounding the subject table of the imaging device. This extension could be confluent to the BSL

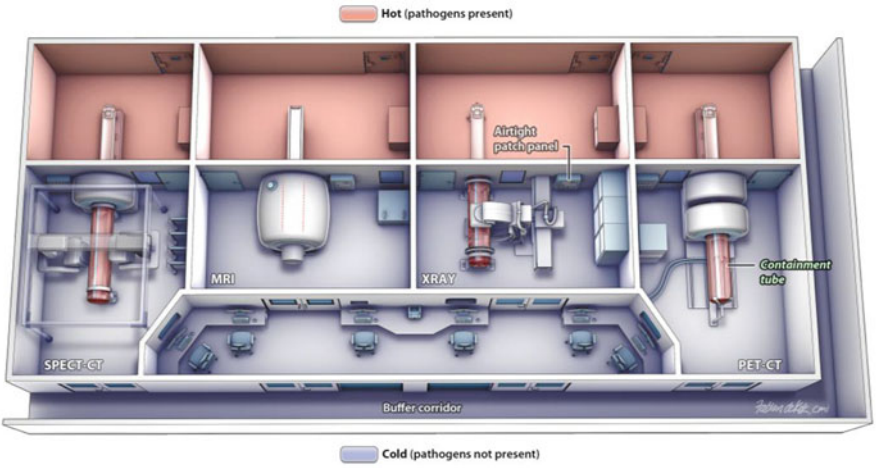

Fig. 10.1 Schematic of medical imaging suite with clinical SPECT/CT, MRI, X-ray, and PET/CT systems integrated into a biosafety level-4 laboratory using the containment tube strategy. The barrier wall and the containment tube separate the high-containment areas (*mauve*) from the non-containment rooms (*blue*). All rights reserved. Reprinted with permission from the American Biological Safety Association (ABSA), Mundelein, IL. Originally published in *Applied Biosafety: Journal of the American Biological Safety Association, 16*(2), p. 59. Copyright © 2011. Visit http://www.absa.org for more information

laboratory, as in the containment tube strategy that will be discussed below, or could involve an encapsulation of the infected subject (Jain et al. 2010).

The National Institute of Allergy and Infectious Diseases Integrated Research Facility (IRF) at Fort Detrick, MD, pursued a biocontainment extension design that located only those imaging elements that are absolutely necessary inside the high-containment zone. The IRF is equipped with clinical SPECT/CT, PET/CT, diagnostic X-ray, and MRI systems. The high-containment zone is extended into the bore of each imaging device with the use of transparent biocontainment tubes made of polycarbonate resin thermoplastic (Philips Bioshield™, North Ryde, Australia) (Fig. 10.1) (de Kok-Mercado et al. 2011). The subject is placed on the imaging table in the high-containment side of the barrier wall that then advances into the biocontainment tube prior to imaging. The bulk of the imaging device and associated electronic equipment remain outside of high containment and are therefore accessible to service engineers under normal maintenance and repair conditions. All electrical signals necessary for the imaging modality are sent through an airtight patch panel to the control room on the cold side of the barrier wall (Fig. 10.2). In addition to providing biocontainment, this containment extension tube strategy protects the imaging equipment from gases and chemicals that are used to decontaminate the high-containment laboratory, such as formaldehyde gas, hydrogen peroxide vapor, or chlorine dioxide gas. However, since placing the subject on the imaging table requires access to the hot zone, this containment strategy requires the imaging systems to be dedicated to infectious disease imaging.

Fig. 10.2 Airtight patch panel through which electrical signals are sent to electronic components on the cold side of the barrier wall. This eliminates the need for electronic components to be on the hot side and subjected to decontamination chemicals

The BSL-4 environment itself, along with the containment strategy chosen, will introduce issues unique to each imaging modality. Some of the issues that pertain to the containment extension tube strategy are discussed, and solutions are explored below for SPECT/CT, C-arm diagnostic X-ray, and MRI.

10.3.2.1 SPECT/CT System

The containment extension tube integrated into the SPECT/CT imaging equipment (Fig. 10.3) is 0.6-cm thick with an outer diameter of 61.0 cm. In nuclear imaging with parallel-hole collimators, spatial resolution degrades with increasing distance between source and collimator (Cherry et al. 2003). In a typical clinical environment, one can optimize spatial resolution by minimizing the distance between patient and collimator. With the containment tube strategy, this option is no longer available since the radius of the circular SPECT orbit is restricted to 33.0 cm or larger. The standard low-energy high-resolution (LEHR) collimator routinely used in the clinic is expected to provide inadequate spatial resolution at this operating distance. To overcome this limitation, the manufacturer of the SPECT scanner custom-designed a unique "ultra-ultra high-resolution" (UUHR) collimator for use at the IRF. Characteristics of the UUHR and LEHR collimators are listed in Table 10.1 (Leyson et al. 2012). System spatial resolution is expressed as full width at half maximum of the line spread function of data acquired from imaging Tc-99m-filled capillary tubes. Spatial resolution degradation with increasing distance from the collimator is less severe with the UUHR collimator than with the LEHR collimator (Fig. 10.4).

Fig. 10.3 Photograph of the SPECT/CT equipment with containment extension tube

Table 10.1 Characteristics of the UUHR and LEHR collimators

	UUHR	LEHR
Hole size (flat-to-flat)	1.22 mm	1.22 mm
Hole length	48 mm	27 mm
Septa thickness	0.15 mm	0.15 mm
System spatial resolution at 10 cm distance*	5.3 mm	7.3 mm
Sensitivity*	56 cpm/mCi	168 cpm/mCi

*Data presented were measured with 140 keV photons and 5/8″ NaI(TI) crystal; *cpm* counts per minute

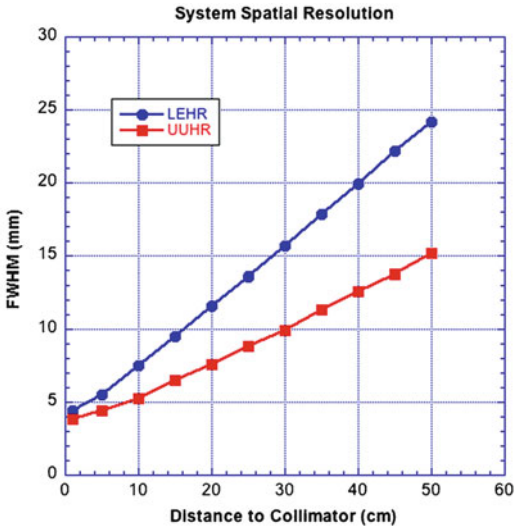

Fig. 10.4 System spatial resolution expressed as full width at half maximum of line spread functions versus distance to collimator using standard low-energy high-resolution (LEHR) and ultra-ultra high-resolution (UUHR) collimators

Fig. 10.5 Diagnostic X-ray installed with containment extension tube

With the UUHR collimator, system resolution at a source-to-collimator distance of 35 cm, an orbital radius that allows room for the biocontainment tube, is comparable to the resolution achieved at 20 cm with the standard parallel-hole LEHR collimator. However, the improved spatial resolution capabilities of the UUHR collimator come at the expense of a substantially reduced planar sensitivity that is nearly one-third that of standard collimators. The reduced sensitivity may be compensated for by administration of a higher dose of injected radioactivity or by a longer acquisition time than required with the LEHR collimator.

10.3.2.2 Diagnostic X-Ray

The implementation of the containment tube strategy into diagnostic X-ray imaging is relatively straightforward (Fig. 10.5). However, one important consideration is the potential for collision between the C-arm gantry and the containment tube that increases the risk of a biocontainment breach. One strategy for collision prevention is to use optical sensors to immediately stop all motion of the device if an object interrupts the optical laser light beam. Such sensors prevent collision of the imaging device with the containment tube as well as the wall of the high-containment laboratory (Fig. 10.6) (de Kok-Mercado et al. 2011).

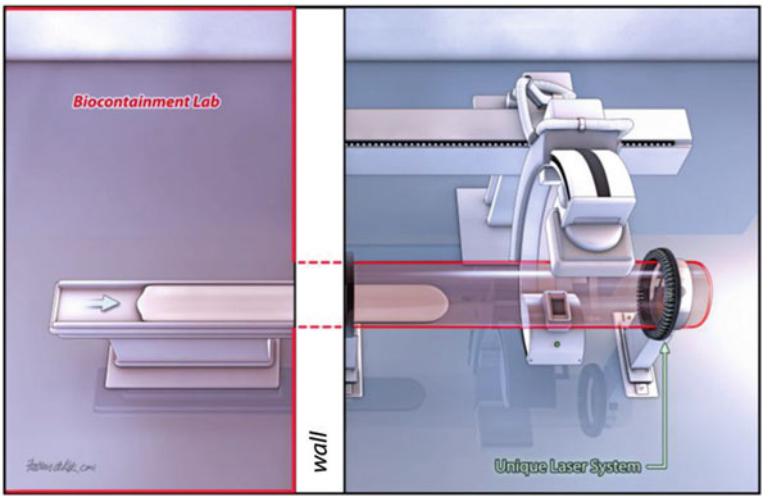

Fig. 10.6 X-ray gantry with its containment tube and unique laser system (black collar around containment tube) to prevent the movable X-ray head from striking the tube as its position is adjusted. If the X-ray head interrupts any laser beam, the power is immediately shut off. The barrier wall and containment tube separate the equipment from the laboratory. All rights reserved. Reprinted with permission from the American Biological Safety Association (ABSA), Mundelein, IL. Originally published in *Applied Biosafety: Journal of the American Biological Safety Association, 16*(2), p. 60. Copyright © 2011. Visit http://www.absa.org for more information

10.3.2.3 Magnetic Resonance Imaging

MRI presents a unique challenge when it comes to the integration of the modality with infectious disease research at high biosafety levels (Fig. 10.7). Even with the implementation of the containment extension tube strategy, MRI can require imaging equipment to be housed and operated within the containment zone. Most commercial MR scanners come equipped with a body radio frequency (RF)-receiver coil housed within the main bore, which is outside the containment tube in the containment extension strategy described above. In contrast, external RF-receiver coil arrays can appreciably improve signal-to-noise ratio and decrease scan time by taking advantage of an accelerated MR acquisition strategy called parallel imaging (Blaimer et al. 2004). Specialized external RF coils dedicated to a specific anatomical region or imaging protocol are commonly used. However, clinical RF-receive coils are costly and are not designed to withstand the autoclaving process associated with removal from biocontainment should repair be required. Possible solutions to repairing RF coils include use of off-the-shelf coils that can sustain the decontamination process or are inexpensive to replace should the coils become unusable (Dannels et al. 2008). Alternatively, custom, in-house coils could be designed such that coil maintenance can be performed in the biocontainment suite. Another option is the reliance on the body coil if external RF coils are damaged or worn.

Fig. 10.7 High-containment side of the magnetic resonance imaging room at the National Institute of Allergy and Infectious Diseases Integrated Research Facility at Fort Detrick, MD. The *black ring* around the bore entrance is a gasket that seals the connection between the containment tube and the barrier wall

Beyond issues with the MRI equipment, the associated strong magnetic field introduces a unique, additional hazard into a high-containment facility. The chance of ferrous objects entering the magnet room is always a risk in clinical MRI. Typically, the danger with this scenario is injury due to ferrous objects flying into the bore of the magnet. While this danger remains present with high-containment MRI, an additional risk of biocontainment breach is possible due to missile objects. Extreme care and caution must be used to avoid these dangerous events (Chaljub et al. 2001; Zimmer et al. 2004).

10.4 Conclusion

Regardless of the challenges involved with integrating medical imaging equipment into high-containment laboratory spaces, structural and functional imaging could serve as an integral tool in the development of therapeutics against highly pathogenic infectious diseases. Using nuclear imaging probes that are currently available, SPECT and PET imaging can identify foci of increased cell metabolism or proliferation after infection. The development of a greater number of more specialized and specific probes would make these modalities even more influential. Certain MRI techniques have the advantage of not requiring administration of exogenous contrast agents, which decreases risk of exposure from accidental needle sticks. Also advantageous is the possibility of cell

labeling and tracking with SPIOs or USPIOs as contrast agents. New, meaningful image biomarkers are being developed in animals to characterize infectious disease processes and efficacy or safety of a new drug. However, care must be taken to validate these imaging biomarkers thoroughly prior to implementation.

Following image acquisition, standard image analysis processes must be optimized for imaging experimentally infected small animals in longitudinal studies in a high-containment laboratory. Such optimization in part will require the development of new, small animal atlases for automated segmentation algorithms as well as rigorous image registration routines. As positioning of infected small animals is hampered by personal protective equipment required for the safety of laboratory personnel, image registration methods must be utilized in order to minimize larger positional differences in subject orientation across scanning sessions.

Numerous strategies are available to physically integrate and install medical imaging equipment into BSL-3 and BSL-4 laboratory spaces. The advantages and disadvantages associated with each strategy may differ depending on the requirements of each facility. An assessment of the laboratory necessities is recommended for new high-containment laboratories. As a result of such assessment and deliberation, personnel from the Integrated Research Facility in Fort Detrick, MD, elected for the containment tube strategy described above. With the containment tube strategy, repair of imaging equipment does not require entry into high containment. The containment tube assures the safety of imaging and repair personnel by preventing exposure to highly pathogenic agents.

By using the same imaging techniques throughout the drug development process, the translation from basic science biodefense research to clinical evaluation will be streamlined in a way that could decrease the approval time for an investigational agent. Imaging biomarkers identified in animals could be used as surrogate markers in clinical evaluation. Image analysis techniques used to track progression of viral infection and therapeutic response to a drug candidate could also be applied to tracking response of patients to a therapeutic intervention. The integration of temporal, spatial, functional, and metabolic information gained from medical imaging modalities in combination with standard evaluation of antiviral drug candidates will have a great impact on the course of future drug development.

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References

Bading JR, Shields AF (2008) Imaging of cell proliferation: status and prospects. J Nucl Med 49(Suppl 2):64S–80S

Beckmann N et al (2003) Macrophage infiltration into the rat knee detected by MRI in a model of antigen-induced arthritis. Magn Reson Med 49(6):1047–1055

Bellani G et al (2010) Imaging of lung inflammation during severe influenza A: H1N1. Intensive Care Med 36(4):717–718
Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95
Blaimer M et al (2004) SMASH, SENSE, PILS, GRAPPA: how to choose the optimal method. Top Magn Reson Imaging 15(4):223–236
Bray M et al (2010) Radiolabeled antiviral drugs and antibodies as virus-specific imaging probes. Antiviral Res 88(2):129–142
Brunetti A et al (1989) Reversal of brain metabolic abnormalities following treatment of AIDS dementia complex with 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine): a PET-FDG study. J Nucl Med 30(5):581–590
Brust D et al (2006) Fluorodeoxyglucose imaging in healthy subjects with HIV infection: impact of disease stage and therapy on pattern of nodal activation. AIDS 20(7):985–993
Bulte JW (2009) In vivo MRI cell tracking: clinical studies. Am J Roentgenol 193(2):314–325
Burton JM et al (2004) Neurological manifestations of West Nile virus infection. Can J Neurol Sci 31(2):185–193
Buscombe JR, Oyen WJ, Corstens FH (1995) Use of polyclonal IgG in HIV infection and AIDS. Q J Nucl Med 39(3):212–220
Chaljub G et al (2001) Projectile cylinder accidents resulting from the presence of ferromagnetic nitrous oxide or oxygen tanks in the MR suite. Am J Roentgenol 177(1):27–30
Cherry SR, Sorenson JA, Phelps ME (2003) The gamma camera: performance characteristics. In: Physics in nuclear medicine. Saunders, Philadephia, PA
Cyran CC et al (2012) Permeability to macromolecular contrast media quantified by dynamic MRI correlates with tumor tissue assays of vascular endothelial growth factor (VEGF). Eur J Radiol 81(5):891–896
Dannels WR et al (2008) Integrated system of MRI RF loop coils plus spacing fixtures with biocontainment tubes. WIPO Patent Application. PCT/US2008 073512, filed June 19, 2008
de Kok-Mercado F, Kutlak F, Jahrling PB (2011) The NIAID integrated research facility at Fort Detrick. Appl Biosafety 16(2):58–66
Di Mascio M et al (2009) Antiretroviral tissue kinetics: in vivo imaging using positron emission tomography. Antimicrob Agents Chemother 53(10):4086–4095
Dotti G et al (2009) Repetitive noninvasive monitoring of HSV1-tk-expressing T cells intravenously infused into nonhuman primates using positron emission tomography and computed tomography with 18F-FEAU. Mol Imaging 8(4):230–237
Dyall J et al (2011) Evaluation of monkeypox disease progression by molecular imaging. J Infect Dis 204(12):1902–1911
Dyall J et al (2012) Assessment of longitudinal changes in 18FDG uptake in bone marrow and lymph node and in lymph node volume in nonhuman primate model of monkeypox infection. World Molecular Imaging Congress Dublin, Ireland, Sep 5–8 2012
Ferro-Flores G, Ocampo-Garcia BE, Melendez-Alafort L (2012) Development of specific radiopharmaceuticals for infection imaging by targeting infectious micro-organisms. Curr Pharm Des 18(8):1098–1106
Floris S et al (2004) Blood-brain barrier permeability and monocyte infiltration in experimental allergic encephalomyelitis: a quantitative MRI study. Brain 127(Pt 3):616–627
Frank JA et al (2003) Clinically applicable labeling of mammalian and stem cells by combining superparamagnetic iron oxides and transfection agents. Radiology 228(2):480–487
Gambhir SS et al (2000) Imaging transgene expression with radionuclide imaging technologies. Neoplasia 2(1–2):118–138
Garg M et al (2008) Radiolabeling, pharmacoscintigraphic evaluation and antiretroviral efficacy of stavudine loaded 99mTc labeled galactosylated liposomes. Eur J Pharm Sci 33(3):271–281
Haacke EM et al (2004) Susceptibility weighted imaging (SWI). Magn Reson Med 52(3): 612–618
Hamed IA et al (1979) Pulmonary cytomegalovirus infection: detection by Gallium 67 imaging in the transplant patient. Arch Intern Med 139(3):286–288

Hayes EB, Gubler DJ (2006) West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. Annu Rev Med 57:181–194
Hoehn M et al (2007) Cell tracking using magnetic resonance imaging. J Physiol 584(Pt 1):25–30
Jain NK et al (2010) Biosafety level (BSL)-3 life support cell for studying live animals. Patent Application. PCT/US20100313821, filed June 16, 2010
Jeha LE et al (2003) West Nile virus infection: a new acute paralytic illness. Neurology 61(1):55–59
Jubeli E et al (2012) E-selectin as a target for drug delivery and molecular imaging. J Control Release 158(2):194–206
Kleinschnitz C et al (2003) In vivo monitoring of macrophage infiltration in experimental ischemic brain lesions by magnetic resonance imaging. J Cereb Blood Flow Metab 23(11):1356–1361
Kumar V (2005) Radiolabeled white blood cells and direct targeting of micro-organisms for infection imaging. Q J Nucl Med 49(4):325–338
Kumar S et al (1997) MRI in Japanese encephalitis. Neuroradiology 39(3):180–184
Kumar M et al (2010) Image-guided breast tumor therapy using a small interfering RNA nanodrug. Cancer Res 70(19):7553–7561
Leyson C et al (2012) Performance comparison of ultra-ultra high resolution collimators to low-energy high resolution collimators. J Nucl Med 53(suppl 1):2409
Liu N et al (2007) Radiolabeling small RNA with technetium-99m for visualizing cellular delivery and mouse biodistribution. Nucl Med Biol 34(4):399–404
Liu T et al (2012) Cerebral microbleeds: burden assessment by using quantitative susceptibility mapping. Radiology 262(1):269–278
Lobel U et al (2010) Three-dimensional susceptibility-weighted imaging and two-dimensional T2*-weighted gradient-echo imaging of intratumoral hemorrhages in pediatric diffuse intrinsic pontine glioma. Neuroradiology 52(12):1167–1177
Luker GD, Sharma V, Piwnica-Worms D (2003) Visualizing protein-protein interactions in living animals. Methods 29(1):110–122
Modo M, Hoehn M, Bulte JW (2005) Cellular MR imaging. Mol Imaging 4(3):143–164
Nahrendorf M et al (2006) Noninvasive vascular cell adhesion molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis. Circulation 114(14):1504–1511
O'Connor JP et al (2007) DCE-MRI biomarkers in the clinical evaluation of antiangiogenic and vascular disrupting agents. Br J Cancer 96(2):189–195
O'Connor JP et al (2008) Quantitative imaging biomarkers in the clinical development of targeted therapeutics: current and future perspectives. Lancet Oncol 9(8):766–776
Padhani AR et al (2000) Dynamic contrast enhanced MRI of prostate cancer: correlation with morphology and tumour stage, histological grade and PSA. Clin Radiol 55(2):99–109
Penheiter AR, Russell SJ, Carlson SK (2012) The sodium iodide symporter (NIS) as an imaging reporter for gene, viral, and cell-based therapies. Curr Gene Ther 12(1):33–47
Pien HH et al (2005) Using imaging biomarkers to accelerate drug development and clinical trials. Drug Discov Today 10(4):259–266
Ratai EM et al (2010) Proton magnetic resonance spectroscopy reveals neuroprotection by oral minocycline in a nonhuman primate model of accelerated NeuroAIDS. PLoS One 5(5):e10523
Reinders Folmer SC et al (1986) Gallium-67 lung scintigraphy in patients with acquired immune deficiency syndrome (AIDS). Eur J Respir Dis 68(5):313–318
Richter WS (2006) Imaging biomarkers as surrogate endpoints for drug development. Eur J Nucl Med Mol Imaging 33(Suppl 1):6–10
Rojas JJ, Thorne SH (2012) Theranostic potential of oncolytic vaccinia virus. Theranostics 2(4):363–373
Rubin RH, Fischman AJ (1994) The use of radiolabeled nonspecific immunoglobulin in the detection of focal inflammation. Semin Nucl Med 24(2):169–179
Rudin M, Weissleder R (2003) Molecular imaging in drug discovery and development. Nat Rev Drug Discov 2(2):123–131
Schellingerhout D et al (1998) Mapping the in vivo distribution of herpes simplex virions. Hum Gene Ther 9(11):1543–1549

Sejvar JJ et al (2003) Neurologic manifestations and outcome of West Nile virus infection. JAMA 290(4):511–515
Sharma V, Luker GD, Piwnica-Worms D (2002) Molecular imaging of gene expression and protein function in vivo with PET and SPECT. J Magn Reson Imaging 16(4):336–351
Shoji H et al (1994) Magnetic resonance imaging findings in Japanese encephalitis. White matter lesions. J Neuroimaging 4(4):206–211
Shu CJ et al (2010) Novel PET probes specific for deoxycytidine kinase. J Nucl Med 51(7):1092–1098
Smith JJ, Sorensen AG, Thrall JH (2003) Biomarkers in imaging: realizing radiology's future. Radiology 227(3):633–638
Stoll G et al (2004) In vivo monitoring of macrophage infiltration in experimental autoimmune neuritis by magnetic resonance imaging. J Neuroimmunol 149(1–2):142–146
Umenai T et al (1985) Japanese encephalitis: current worldwide status. Bull World Health Organ 63(4):625–631
US Department of Agriculture (2005) Agriculture bioterrorism protection act of 2002; possession, use and transfer of select agents and toxins, final rule. Fed Regist 70(52):13294
US Department of Health and Human Services, Centers for Disease Control and Prevention, National Institutes of Health (2009) In: Chosewood LC, Wilson DE (eds) Biosafety in microbiological and biomedical laboratories, 5th edn. US Government Printing Office: Washington, DC. http://www.cdc.gov/biosafety/publications/bmbl5/. Accessed 5 Dec 2012
US Food and Drug Administration (2002) New drug and biological drug products; evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible. Fed Regist 67(105):37988–37998
Valadon P et al (2006) Screening phage display libraries for organ-specific vascular immunotargeting in vivo. Proc Natl Acad Sci USA 103(2):407–412
Venneti S et al (2004) PET imaging of brain macrophages using the peripheral benzodiazepine receptor in a macaque model of neuroAIDS. J Clin Invest 113(7):981–989
Viglianti BL et al (2004) In vivo monitoring of tissue pharmacokinetics of liposome/drug using MRI: illustration of targeted delivery. Magn Reson Med 51(6):1153–1162
Wanahita A et al (2007) Diagnostic sensitivity and specificity of the radionuclide (indium)-labeled leukocyte scan. J Infect 55(3):214–219
Wang YX, Deng M (2010) Medical imaging in new drug clinical development. J Thorac Dis 2(4):245–252
Willmann JK et al (2008) Molecular imaging in drug development. Nat Rev Drug Discov 7(7):591–607
Yarchoan R et al (1987) Response of human-immunodeficiency-virus-associated neurological disease to 3′-azido-3′-deoxythymidine. Lancet 1(8525):132–135
Zimmer C et al (2004) Near-miss accident during magnetic resonance imaging by a "flying sevoflurane vaporizer" due to ferromagnetism undetectable by handheld magnet. Anesthesiology 100(5):1329–1330