DEPARTMENT OF BIO SCIENCE &BIOTECHNOLOGY

For the award of the degree of

MASTER OF SCIENCE IN BIO TECHNOLOGY

Submitted by

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M Sc (BIO TECHNOLOGY)

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DECLARATION

I HEREBY, DECLARE THAT THE SUBJECT MATTER EMBODIED IN THIS PROJECT REPORT ,ENTITLED **"PROSTATE CANCER"** WHICH BEING SUBMITTED BY ME, FOR DEGREE OF THE MASTER OF SCIENCE IN BIO TECHNOLOGY. KRISHNA UNIVERSITY, MACHILIPATNAM ,(A.P),INDIA, IS THE RESULT OF RESEARCH BY ME UNDER THE GUIDANCE OF **DR. JN. LAVANYA LATHA** ASSOCIATE PROFFESSOR , DEPARTMENT OF BIO SCIENCE & BIO TECHNOLOGY . KRISHNA UNIVERSITY, MACHILIPATNAM

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CERTIFICATE

This is to certify that the project report entitled "PROSTRATE CANCER" is a bonafied work carried out by P.SHIVA NAGA GANESH (Y21BIT101019) under my supervision is submitted in partial fulfillment of the requirements for the award of degree of Master of Science Bio Science &Bio Technology to Krishna University, Machilipatnam , A.P. No part of the dissertation has been submitted for any degree/diploma or any other academic award any where before.

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Examinedon:……

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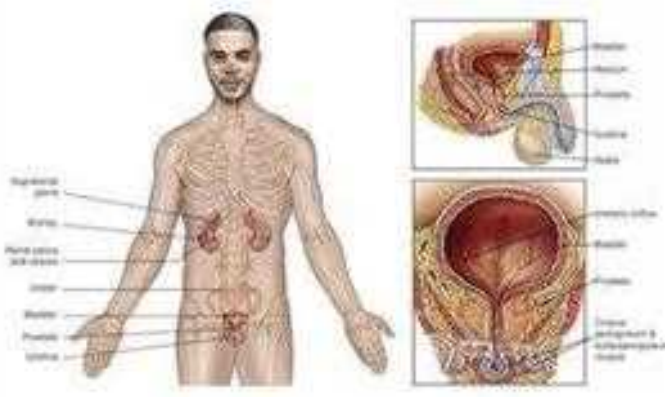
Abstract

The use of genetically modified animals has been studied in scientific research over time as away to discover new treatments or even a cure for various diseases. Transgenic Adeno carcinoma of the Mouse Prostate (TRAMP) is a model for prostate cancer (PCa) that develops lesions that range from preneo plastic to metastasis. Its similarity to human PCa brings essential knowledge about disease development as well as making possible to investigate different degrees of the tumor profile. We reviewed the literature regarding five important areas relating to PCa progression in the TRAMP model. We also present some useful PCa models comparing them to TRAMP. Furthermore, we investigated the effect of some therapies related to these areas highlighting the best approaches that can delay PCa progression. The revised studies showed that TRAMP cancer stages are well established from 8 to 30 weeks of age, which makes possible to interfere in specific times of PC adevelopment. Moreover,inflammatory andangiogenic blockage before the appearance of malignantlesions retarded PC aprogression and showed better results than therapeutical approaches in other phases in TRAMPmice. Reactive stromaisless studied than other areas, although thas been showing a particular relevance in PC a asamilestone inmalignant transformation through the modulation of TGF-β,vimentin, and α SMA .We concluded that even years after its creation, the TRAMP model is still one of the most essential tools for PCa study, as well as for the development of new strategies to prevent the disease.

Introduction

For almost 25years, the term TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) has been present in a wide variety of studies to design at easpecific genetically engineered mouse.This model re-sembles human prostate cancer (PCa) features, showing dierent lesion grade sandprogression in a short period. Thus, researcher shaveex-plored several investigative methodologies in this model to understandand uncover the complex process involved in PCa. TRAMP mice were engineer edusing the minimal ratprobasin promoter (−426/+28) to target the largeTSV40virus expression and small to ncoproteins, only in the prostatic epithelium, in activating p53 and Rb tumor suppressor activity in the prostate. As a result,this model is able to develop not only progressive PCa, but also androgen independence and metastatic spread. The advantage of monitoring prostaticlesion progression in a short time has brought relevant results for TRAMP studies, including morphological characterization of prostatic tissue and other organs cancer metabolism diagnosis prevention treatment and novel drug discovery. According to Balmain, the use of mouse models shows advantages since it allows the control of aspects such as environmental exposure that leads to cancer onset, which is as yet not possible in human beings.Further more,the genetically engineered mice (GEM) have contributed to elucidate dierent biological processes involved in aggressive profile PC anotonly in basic but also translational research .However, it is important to make clear that until now no single mouse model comprises all features of human PCa progression,there fore many models have been created to reproduce the main characteristics of this disease.



sund Information

Historically, researcher shavereliedoncul-turedtumorcelllines for testing immuno-therapeutic approaches to treating prostate can-cer . This entailed the propagation of cells invitro and the sub sequent implantation of these cells into syngeneic rodents, most notably the transfer of rat Dunning prostate tumor cells into syngeneic Copenhagen rats to promote tumour . An alternate approach was the use of spontaneous or chemical carcinogenesis models that are associated with a relatively lower incidence of tumors and pro-longed interval to tumor formation how-ever, the introduction of transgenic technology in the 1980s laid a critical cornerstone for the subsequent development of novel models that recapitulate primary tumor formation in a more controlled and relevant fashion.

To create one of the first transgenic tumormodels , Hanahan (1985)placedanSV40largeT antigen transgene under the transcriptional control of the rat insulin promoter. This promoter directs nearly tissue-specific expression of the viral transforming antigen by the bis let cells of the pancreas, with some promiscuity of TAg expression in the thymus . Consequently, these transgenic “RIPTAg” mice develop carcinomas of the pancreas.It is anticipated that newer tissue-specific, tumor suppressor, gene knock-out models will provide even further refine mentis transgenic model technology to support future investigations into the pathogenesis and treatment of cancer.

The TRAMP model is comparable to the RIP TAg model, with the added feature that on cogenesis is developmentally regulated . The TRAMP model was generated using the minimal probasin (PB)-426/+28 regulatory sequence to restrict SV40 early gene (T and t antigens; TAg) expression to prostatic epithelium in a developmentally and hormonally regulated fashion (Greenbergetal. In TRAMP mice, expression of the transgene can be detected as early as 3 weeksof age (i.e., at the onset of puberty) and is maximal by the time the mice are ~12 weeksof age. Over a 30 week period, 100% of the male TRAMP mice will develop prostate disease. Disease pathogenesis in this model mirrors that observed in humans, evolving through the early PIN (prostatic intraepithelial hyper-plasia) lesion between 8 and 12 weeks of age, well differentiated adeno carcino masat 12 weeks of age, moderately differentiated carcinomas by 18 weeks of age, and poorly differentiated carcinomas by 24 to 30 weeks of age . Distant metastases, both hematogenous and lymphatic, have also been shown to develop as early as 12 weeks of age but are most prevalent by the time mice reach24 to 30 weeks of age . Additionally, TRAMP provides a model for androgen insensitive disease progression. In support of this, following castrationat 6weeks of age,~50% of TRAMPmice will ultimately experience androgen-independent disease progression however, consistent with progressive but stochastic acquisition of secondary molecular events driving further transformation, ~80% of TRAMP males castratedat 12weeks of age will ultimately suffer androgen-independent disease progression.Finally, castrated mice uniformly develop poorly-differentiated adeno carcinomas during androgen-independent progression .

Since TRAMP mice are born disease-freebut are ultimately genetically predisposed to develop prostate cancer, the semice provide an ideal model for testing novel strategies related to prostate cancer prevention and therapy. Recently, the TRAMP model was used to evaluate the ability of E-7869(R-flurbiprofen)to inhibit prostate cancer progression .E-7869is an enantiomerofanonsteroi-dal anti inflammatory drug that does not inhibit Cox-1 or Cox-2. In this study, TRAMP mice were treated with E-7869 for 18 weeks. Remarkably, the urogenital and lymph node wet weights were significantly lower in the mice receiving E-7869 at adose of 20mg/kg . These results serve to illustrate two important points. First, that E-7869 is a promising chemopreventive agent for human prostate cancer and second, that even a genetically engineered transgenic model suchas TRAMP, which is indisputably a virulent model for cancer progression, is amenable to therapeutic intervention. Studies

of this nature strongly support the validity of auto chthonous models like TRAMP for preclinical testing and development of preventive cancer interventions.

As a further extension of the TRAMP model system, several TRAMP tumor derived poly clonal tumor cell lines (TRAMP-C) have been established and characterized .These lines are tumorigenic in wild type C57BL/6 mice and express low but detectable levels of MHC antigens that can be up regulated invitro following stimulation within terferong. Interestingly, using molecular analysis we have shown that these lines,whether grown invitro orasinvivo tumors, have lost the ability to expressTAg . This loss of TAg expression is furthe rsupported by recognition and killing of TRAMP-C cells by allo reactive cytotoxic T lymphocytes (CTLs)but not TAg specific CTLs.

Using the TRAMP-C1 cell line we initially reported that in vivo anti body mediated blockade of T cell inhibitory CTLA-4/B7 interactions promotes regression of un modified TRAMP-C1 tumors . This rejection of TRAMP-C1tumors is dependent on both T cells and NK cells and can provide protective immunity against subsequent tumor cell challenge. In addition, weal so reported that conferral of B7.1(CD80) expression to TRAMP-C1 cells by genetic modification is sufficient to promote regression of TRAMP-C1 cells in a T cell dependent mechanism .B7.1is the antigen specific co stimulatory mole cule/ligand required for the induction of T cell activation.

More recently, Ciavarra and colleagues (2000) demonstrated that TRAMP-C1 tumor growth can be slowed by systemic administration of flt-3 ligand protein which serves to enhance anti tumor priming by promoting host dendritic cell expansion. Unfortunately, this effect is lost upon withdrawal of flt-3 ligandand thus, it appears that short-term flt-3 administration does not elicit protective immunity against the TRAMP-C1 tumors.

Finally, the metastatic capability of the TRAMPC-2 cell line has provided a unique minimal residual disease model for testing the effectiveness of various the rapies against pre established metastases after surgical removal of the primary tumor. Using the TRAMP-C2 tumor resection /metastasis model, we have shown that CTLA-4 blockade (provided by administration of an anti-CTLA-4 anti body that blocks interactions between CTLA-4 andB7), given immediately after tumor resection, can diminish the rate of post surgical metastatic failure by 50%. Hence, the TRAMP-C tumor lines have certainly proved useful as targets for screening prostate cancer immunotherapy's however, their most effective immunologic application may be as reagents to construct vaccines for the treatment of autochthonous TRAMP tumors.

Perhaps one of the most rigorous applications of the TRAMP system to date has beenthe testing of experimental immunotherapy's to treat autochthonous tumors in TRAMP mice.Two such studies have been reported. In the first, Vitiello and colleagues tested an adoptive approach to immunotherapy using the TRAMP model . Splenocytes from syngeneic C57BL/6 mice presensitized to TAg-expressing tumor cells were transferred into naïve TRAMP mice. Using three doses of transferred splenocytes administered at 10,16,and 23 weeks of age, the authors reported a significant reduction in TRAMP tumor progression as well as sustained immunity to TAg. Quite surprisingly, the authors noted that transfer of naïve splenocytes from non transgenic C57BL/6 mice into TRAMP mice also results in“partial”protection.These findings suggest that while TRAMP mice develop tolerance to TAg, naïve mice apparently possess the capability of mounting an innate, antitumor response with little sensitization. Nevertheless, sensitization to TAg profoundly enhances immunity .Recently, we have detected TAg mRN expression in the

thymus of mice age between 8 and 15 weeks, suggesting that central tolerance may play a role in generating tolerance to prostate tumor associated antigens in TRAMP mice.

In a more recent report, we demonstrated that treatment of TRAMP mice with anti-CTLA-4 and a cell based vaccine composed of amixture of irradiated TRAMP-C1 and TRAMP-C2 cells transduced to express granulocyte macrophage colony stimulating factor GM-TRAMP-C, can reduce the incidence and severity of prostatic tumors in TRAMP mice . In this study, mice were treated at~15weeks of age and tumor progression assessed 8 weeks later. Interestingly, the most prominent histologic feature noted in this study was the presence of inflammatory foci in the interductal spaces of the prostatic acini (highly reminiscent of prostatitis) of TRAMP mice treated with a combination of anti-CTLA-4 and GM-TRAMP-C vaccine cells. Similar treatment of wild-type C57BL/6 mice also resulted in the development of prostatitis with in these non transgenic animals . Given these latter findings, it would appear that the antitumor response raised by our experimental immunotherapies is at least,in part, directed against normal prostate anti-gens. Most impressive in these two studies isthe ability of the immune system to stem the worsening of prostate disease in a model that is so strongly programmed to undergo progressive neo plastic transformation. Thus, TRAMP mice provide one of the few animal models of primary tumor development that is amenable to immunotherapeutic manipulation. Identification and estimation of prostate tumors is difficult when mice are younger than12 weeks. Somestudies have attempted to use MRI as a means of identifying enlargement of the prostatic complex however, access to the appropriate imaging instrumentation remains prohibitive. Another alternative may be the development of novel mouse strains that carry readily identifiable genes (e.g.,luciferase or GFP)under a similar, prostate specific promoter. This might facilitate transcutaneous visualization of prostatic tumors in situ. Unfortunately, such strains of mice (which might be crossed onto the TRAMP background)have not yet been generated.

Critical Parameters and Troubleshooting

With regards to the autochthonous TRAMP model, the most important considerations are familiarity with using this model and study endpoints. To date, since serological markers of murine prostate disease have not been identified (e.g., prostate-specific antigen) and methods for imaging in situ tumors (i.e., MRI) remain cumbersome at best, measurement of disease status typically entails direct examination of the prostate, including an assessment of tumor grade and burden. As alluded to in Support Protocol 2, grading of tumors can be demanding and typically requires some assistance from an individual who has prior experience with histopathologic evaluation of prostate tissues. Similarly, extensive practice with micro dissection of the prostate and related tissuesis imperative. Microsurgical separation of the murine prostate in to its individual lobes is particularly important since normal histology in one of the four paired glands (or the seminal vesicles) can occasionally be confused with a diseased state in another.

If survival is chosen as an end point, one must consider both time constraints(see below) and the ultimate condition of the animal throughout the study interval. To date, all TRAMP mice that we have followed in a “survival” setting have developed prostate pathology. Advanced prostate cancer in these mice can cause considerable cachexia, urinary obstruction, loss of appetite, paralysis, and ultimately death for all mice. While many of these mice harbor large

primary tumors, a small proportion of mice will experience rapid deterioration in their health due to extensive metastases and not primary disease. Hence, it is advisable to follow these mice closely and, most importantly, to establish strict criteria for euthanasia so as to avoid "sliding scale" criteria after initiating a survival study.

Related to these points, it is important to consider appropriate cohort-size. Based on our early experience with~300TRAMPmice, we have determined that ~25 mice per treatment cohort would be required to identify a statistically significant 15 per cent difference in mean tumor size with in an 80% confidence interval. This requirement for large cohorts emanates from sizable stochastic differences in rates of tumor growth in the TRAMP model. Also, to further preclude biases introduced by additional factors (e.g., litter, and box effect), we recommend that cohorts be equally represented by age- and litter-matched animals. Finally, it is important to remember that characteristics of tumor formation difference between pure C57BL/6TRAMP and C57BL/6TRAMP´FVB/N

($TRAMPF_1$)mice, and further differences might be anticipated without breeding into other distinct strains of mice.

For the subcutaneous models described (see Basic Protocol 2 and Alternate Protocol), the main points to consider relate to the fastidious maintenance and handling of the TRAMP-C cell lines. We previously established that the TRAMP-C cell lines tested negative for 12 different murine viral pathogens by mouse antibody production (MAP) assay. These lines are routinely tested for mycoplasma at regular in-tervals in our laboratory. To reduce potential contamination by fetal calf antigens, as with any cell line, it is advisable to wash TRAMP-C cells with liberal amounts of PBS or plain media (three times with ~5 ml/10^6 cells) prior to their injection into mice. Finally, unless otherwise intended, TRAMP-C cells should be injected into the syngeneic male C57BL/6host.We previously determined that injection of these cells into normal female C57BL/6 mice fails to produce tumors, where as injection into female C57BL/6 nu/nu or severe combined immunodeficient (SCID) mice results in anticipated rates of TRAMP-C tumor outgrowth. Based on this experience, we surmise that TRAMP-C cells fail to proliferate in the intact female due to immunologic rejection and not hormone-related influences.

The TRAMP Mouse as a Model for Prostate Cancer :

Prostate cancer ranks as one of the most common cancers among men. Consistent with this, prostate cancer is also one of the most frequent causes of male cancer-related death. Adenocarcinoma represents the predominant histologic type of prostate cancer, typically arising from malignant transformation of luminal epithelial cells within glands comprising the peripheral zone of the prostate. Somewhat unique to this cancer are a number of key features. Androgens play an essential role not only in normal prostate development but also the pathogenesis of prostate cancer. Related to this, most prostate tumors are "androgen-sensitive"—i.e., demonstrate initial regression following androgen removal or blockade, and then subsequent progression due to proliferation of "androgen-inde-pendent" or "androgen-suppressed" tumor cells. Human prostate cancer encompasses a wide spectrum of histologic grades ranging from the putative precursor lesion, prostate intraepithelial neoplasia (PIN), to intermediate-grade cancers that recapitulate rudimen-tary glandular formation, or more poorly differentiated cancers that infiltrate the prostatic stroma and its surrounding tissues. Likewise, metastases can be disseminated over a wide distribution, most frequently involving the pelvic lymph nodes, bone and marrow, and (less frequently) the lungs and other viscera.

To date, a number of different rodent prostate tumor models have been described in the literature. These models, including the Dunning rat prostate tumor model (Lubar off et al.,1980;Isaacs et al.,1986),have played an

instrumental role in the elucidation of mechanisms involved in the pathogenesis of prostate cancer as well as the development and testing of experimental treatments including gene- and immune-based therapies. Recently, a unique model, the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, has garnered widespread attention due to its ability to closely mirror disease pathogenesis as seen in man (Greenberg et al., 1995). Unlike prior rodent models, male TRAMP mice uniformly and spontaneously develop autochthonous (orthotopic) prostatetumors following the onset of puberty (see Basic Protocol 1). Prostate cancer occurs consequent to the expression of the oncoprotein, SV40 T antigen (TAg), which is under transcriptional control of the rat probasin promoter. The probasin promoter appears to maintain high specificity, restricting Tag expression to epithelial cells with in the prostate . Another unique feature of TRAMP is that TAg expression is androgen-driven and developmentally regulated. Related to this, autochthonous TRAMPtumors transiently regress following androgen withdrawal (e.g., by castration), but subsequently recur in a high percentage of mice, paralleling the ultimate emergence of fatal androgen-independent prostate cancer as is commonly observed in man.

The versatility of the TRAMP model has been extended further by the establishment of several TRAMP-derived prostate tumor cells lines (including TRAMP-C1 and TRAMP-C2) that can be injected into the syngeneic male nontransgenic C57BL/6 host to induce ectopic prostate tumorigenesis (see Basic Protocol 2).These tumor cell lines can also be genetically manipulated in vitro for establishment of tumor-cell vaccine preparations intended to induce immunologic responses directed against syngeneic TRAMP tumors . Subcutaneous tumor induction using the TRAMP-C cell lines has provided the basis for two additional TRAMP-based murine models, the first of which can be used for rapid screening of experimental the rapies that might prove effective for the treatment of primary prostate tumors (see Basic Protocol 2) and the second for testing the effectiveness of adjunctive therapies targeting prostate cancer metastases (see Alternate Protocol).These models, combined with the

TRAMP model and its derivative cell lines, provide a comprehensive model system that readily lends itself to studies related to prostate cancer. Detailed descriptions for the harvesting and microdissection of TRAMP prostate sand tumors , and the evaluation and scoring of tumors are also provided. Finally, a protocol for androgen withdrawal by castrationis provided for studies intended to examine the biological effects of hormone manipulation as it pertain stop rostate cancer biology and immunotherapy.

THE BREEDING AND SCREENING OF C57BL/6($H\text{-}2^b$) OR C57BL/6´FVB/N(H-2^q)F1 TRAMP MICE

Greenberg et al. (1995) introduced the murine TRAMP model which was originally generated from a single transgenic founder (#8247) in the C57BL/6 background. Het-erozygous postpubertal male progeny from this line developed enocarcinoma,predomi-nantly affecting the paired dorsal and lateral lobes of the murine prostate. Tumor progression in heterozygous TRAMP mice is slow andinsidious, often becoming apparent as a softmass in the lower abdomen of an 18 to 24 week-old mouse. This mass typically results from carcinomatous invasion, consequent dilation of the seminal vesicles, and toa lesser extent obstruction of the bladder. In contrast, male offspring of TRAMP mice back-crossed one generation onto the FVB/N background ($TRAMPF_1$) exhibit a some-what accelerated pattern of tumorigenesis and tend to form firm spherical and vascular tumors arising

from the dorso lateral prostate without significant seminal vesicle invasion(Gingrich et al., 1999). While rates and patterns of metastasis formation are roughly equivalent in these two models, survival for F_1 males (maximal survival <33 weeks) appear stobe shorter than for heterozygous C57BL/6TRAMP males (mean survival~33to40 weeks) owing to aggressive bulky tumor formation in the F_1 males . The accelerated pattern of prostate tumor formation, shorter survival, and larger litter sizes emanating from out breeding has prompted some investigator stofavor the F_1 model for the irstudies. Finally, while homozygous C57BL/6 TRAMP mice are especially useful for breeding and maintaining a TRAMP colony, these mice also appear to experience an accelerated form of disease with commensurate reductions in rates of survival, relative to their heterozygous counterpart, and markedly shortened interval soffertility due to early seminal vesicle invasion by tumors. In the authors' experience, homozygous TRAMP male stend to function as viable breeders for upto~4 to 8 months of age.

PCR is performed as a means of identifying trans mission of the Tag transgene. Two sets of primer sareused, one specificfor *Tag* and one for *casein*. The Tag primers willonly amplify a product if the transgeneis in the germ line DNA. The case in primers serveasa positive control that the PCR reaction is working. Thus, all samples, if successfully prepared, should amplify a case in product. Because this procedure is not quantitative, noconclusions can bedrawnabout Tag copynumber.

Materials

Pure C57BL/6 heterozygous TRAMP colony(Green bergor Jackson Laboratories; see Internet Resources)
6-to 8-week-old male nontransgenicC57BL/6orFVB/N mice (Charles River, Jackson Laboratories, or Taconic)
70%ethanol,4°C
Tailly sis buffer(see recipe)
10mg/ml protein ase Kindi stilled deionized H_2O1:1(v/v)phenol:
chloroform
100%e than ol,4°C
TEbuffer,pH8.0

PCR master mix(see recipe)1%agarosegel

Sharpscissors,clean2-ml tube, sterile55°C incubator
Platform rocker Tablet opcentrifuge

Additional reagents and equipment for anesthesia using methoxy flurane mouse euthanasia .determining DNA concentration and purity by absorptions pectroscopy, and agarosege lelectrophoresis

Breed TRAMP mice

Maintain a pure C57BL/6 heterozygous TRAMP colony.

In order to maintain avigorous C57BL/6TRAMP colony, we generally recommend frequent(~4 month) breedings to generate new young male breeders since rapid prostate tumor progression in these mice can give rise to impairments in fertility (see above) that can ultimately resultin loss of the colony. This, however, is not a major problem if one maintains a TRAMP colony by breeding homozygous TRAMP females against regular

C57BL/6males. At present, no one is authorized to provide TRAMP homozygous mice. Jackson Laboratories (see Internet Resources) might eventually have homozygous mice available for distribution; however, investigators must currently breed their own.

Others as well as ourselves have successfully established homozygous C57BL/6 TRAMP lines that are particularly useful for the breeding of large experimental cohorts in a relatively short period.

Obtain C57BL/6 TRAMP female mice from the colony and cross with either pure C57BL/6 non transgenic males to generate TRAMP mice in the pure C57BL/6 background, or FVB/N non transgenic males to generate $TRAMPF_1$ mice.

$TRAMPF_1$ mice should not be used for further breedings as the impact of additional alterations in the genetic background of these mice on subsequent characteristics of prostate umorformation (i.e., in ciden ceorpathology) is not known.

Extract tail DNA

At~3weeks of age (see Critical Parameters), an esthetize miceusing me thoxyflurane in halant).

Using cleans harp scissors, cut off0.5to1.0cm of mouse tail and place into asterile2-ml tube. Between each tail-cut(i.e., between mice),cleans cissors with70% ethanol to prevent cross-contamination.

Add 0.45ml tail lysisbuffer and 0.05ml of 10mg/ml protein ase Kindd IH_2O to the tube.

Incubate the tail specimen over night in a 55°C incubator while rocking the ly sis/di-gestion preparation over night.

Add 0.5 ml of 1:1 (v/v) phenol: chloroform to the tail ly sate solution. Vortex the preparation gently and then centrifuge samples10minat12,000´*g*,4°C(i.e., until the to paqueous layer is clear).

Transfer the a queous layer(~0.5ml) to a new2-ml tube. Add two volumes(i.e.,1to

1.5ml)cold100%ethan ol to the a queous solution and rock mixture on a platform rocker until the DNA precipitate sou tof solution.

Recover DNA pellet by centrifuging 5min at12,000´*g*,4°C.Wash with cold70% ethanol.

Gently transfer DNA pellet oa clean micro centri fugetu be containing 0.3ml TE buffer,pH8.0.Dissol vefor12 to 48hr at room temperature with a gitation.

Determine purity and concentration by spectrophotometricA_{260}/A_{280}analysis.

Store tail DNA solution at 4°Cup to1month.

Screen for the TRAMP trans gene using PCR

Add1ml tail DNA solution (maximum 0.5 to1.0mg total DNA) to 49ml PCR master mix.

Perform PCR amplify cation using the following thermal cycler profile:

Initial step:	1min	94°C	(denaturation)
30cycles:	1min	94°C	(denaturation)
	2min	60°C	(annealing)
	3min	72°C	(extension)
Final step:	5min	72°C	(extension/hold).

PCR products can be stored in definitely at 4°C.

Analyze 5ml PCR reaction products perlaneona1%a garosegel. Include an appropriate emolecular weight standard in a separate lane.

*The expected size of the trans gene productis 0.6kB,while the expected size of the internal control(M*bC*)is0.5kB.*

SYNGENEIC TRAMP-C1AND-C2SUBCUTANEOUSTUMOR MODEL

Previously, two prostate can cercelllines,TRAMP-C1and-C2,were established from a tumor harvested froma32-week-oldC57BL/6TRAMP male . These lines retain expression of and rogen receptor(AR), E-cadherin, and cy to keratin supporting the irtissue-specific origin from prostate epithet lial cells following malignant transformation. Interestingly, unlike autochthonous TRAMP tumor cells, these lines no long erexpress TAg.Never the less, and per haps owing to this absence, TRAMP-C1 and -C2 are reliably tumorigenic following sub cut an eousin jection into the non transgenic C57BL/ 6male mouse.Morphologically,TRAMP-C1and -C2 form tumors that are histologically similar to advanced tumors in TRAMP mice.These cell lines also provide a convenien treagent for the creation of cell-based vaccine and facilitate the derivation of potential prostate-specificandnonTAg-related antigens that can be used in combination with the TRAMP model for immunologic studies.

Materials

TRAMP-C1or-C2 cell lines (Green berg Laboratory; see Internet Resources) TRAMP tissue culture medium (see recipe)
0.05% (w/v)tryp sin/0.53mMEDTA solution (Life Technologies) Dulbecco's Modified Eagle Medium
6To 8-week-old male non transgenic C57BL/6mice

10-cm tissue culture dishesor75-cm^2 flasks 15-or50-ml conical tube
1-ml syringe and19-to 21-G,1.5-in.hypodermic needle Electric clippers (Oster) and size 40 blade Vernier calipers

Additional reagents and equipment for counting cells on a hemacyto meter and an esthesiausing methoxyflurane

Culture TRAMP-C1 or-C2cells

PropagateTRAMP-C1or-C2 cells as an adherent cell in ein TRAMP tissue culture medium in a 37°C,5%CO_2 incubator using standard 10-cm tissue cultured is hesor75-cm^2 flasks. Feed with 7 to 10 ml of fresh medium every 2 to 3 days. Split 1:6 to1:10 every 3-5days(i.e., when newly confluent).

Treatcellsfor~10 min with0.05%(w/v)trypsin/0.53MEDTA.Transfertoa15-to50-ml conical tube and centrifuge 5 min at 600 ´ *g*, 4°C to precipitate cells. Wash cells with 15ml DMEM at least 3times to remove residual trypsinand FBS.

It is recommended that TRAMP-C cells be harvested before they become densely confluents in ce these cells produce extensive extra cellular matrix proteins that can preclude ecomplete dis aggregation of cells during trypsinization.

Count live (trypanblue–excluding cells) on a hemacy to meter slide (*APPENDIX3B*) and prepare a final cells us pension in DMEM to achieve 2.5´10^6 cells/0.1ml.

Alternatively, concentrations ranging from0.5to10´10^6cells/0.1ml have been successfully used to establish subcutaneous tumors in mice.

For immunological experiments, only use TRAMP cells if viability is greater than 80%,since dead cells could potentially cause unwanted large-scale dissemination of tumor antigen.

Inoculate tumor cells

For each mouse to be injected, draw up0.1ml of cells us pension into a1-ml syringeanda19-to20-G,1.5-in.hypodermic needle.

Anesthetize 6-to8-week-old male non transgenic C57BL/6 mouse with a short-act-ing, inhalable anesthetic (i.e., methoxy flurane; *UNIT 1.4*). Shave injection site with electric clipper sand a size 40blade.

Tent up (i.e., pinch and lift) the shaven skin of each mouse. Insert and direct thesyringe needle into a subcutaneous location (i.e., either over the rump, flank, or hind-limb, or into the dorsal neck region for the experiments described in Al ternate Protocol).Hold the needle tract while removing the needle and continue to pinch the tract closed for 30 sec after injecting the tumor cell suspension to prevent leakage from and seeding of the needle tract.

No tumor formation occurs following the injection of TRAMP-C cells into the syngeneicC57BL/6femalehost.

Estimate subcutaneous tumor area by measuring two, bisecting diameters of the tumor and using the product of the set wo values.

Generally, TRAMP-C tumors are palpable as a small nodule (<10 mm^2) within 2 weeks following inoculation. Tumors are usually readily measurable within 3 weeks and will demon strate rapid, exponential growth thereafter. Most animal protocols will require euthanasia of mice be aringulcer ated tumors or tumor sw hose estimated area exceeds 250mm^2.

TRAMP-C2SUB CUTANEOUSTUMORRE SECTION-METASTASIS MODEL

To facilitate studies more closely focused on prostate tumor metastasis, we recently introduced an immune competent murine model that nominally recapitulates clinical metastatic cancer relapse after primary tumor resection . The estab-lishment of this model is significant because, in general, the development of adjunctive cancer the rapies has been hindered by the absence of such models. This model capitalizeson the capacity of TRAMP-C2 cells to metastasize to regional lymph nodes and other organs following an interval of chronic primary tumor growth. Following primary tumor resection in this model, metastatic recurrence occurs at a high rate (i.e., >95% of mice)and in a predictable pattern, typically with in the axillary and n terior cervically mphnodes draining the primary tumor site.Meta static failure in this model can be readily quantified by measuring overtly appreciable tumor-bearing nodes. These tumor-bearing nodes can be assessed long itudinally, obviating the need to sacrifice animal cohorts at intervals to determine metastatic tumor burden. Metastatic recurrence in thismodelemanatesfromtheoutgrowthofestablishednodalmicrometastasesthatarepresentatthetimeofprimarytumorresectionandhence,metastaticdiseaseprogressionisnotaconsequenceofseeding.Furthermore,thismodelmimicstheclinicalparadigminwhichdistant metastases represent the most dire consequence of surgical treatment failure.Because of these attributes, this model readily lends itself to the testing of adjunctiveregimens for their ability to eliminate minimal residual disease in the form of prostatecancermetastasesafterprimarytumorremoval.

C57BC/6malemicewithTRAMPC-2necktumors(seeBasicProtocol2)Verniercalipers Metzenbaumscissors.

AdditionalreagentsandequipmentforeuthanasiabyCO_2asphyxiation(*UNIT1.8*),removal of the lymphoid organs , paraffin embedding and sectioning andhematoxylinandeos instaining

Establish TRAMP-C2 tumors in the dorsal neck region of normal C57BL/6 malesusingthemethodsandmaterialsdescribedabove.

Injection of tumor cells into this area permits establishment of large tumors that can beeasilyresectedowingtotheexcessofskinintheneckregion.

Permittumorstogrowuntilthebisectingtumorbasedimensionsequal~250mm^2asmeasuredwithvernier calipers.

Anesthetize the mouse with methoxyflurane . Shave the dorsal head,neck,andbackofthemousewithelectricclippersandasize40blade.

Lift the tumor with fingers and stretch the neck-skin taut. Using Metzenbaumscissors,circumferentiallycuttheskinaroundthetumor,removinga0.3-to0.5-cmmargin.Removethetumorenblocwithinitsinvestingtissuesbytakingallfattytissuewiththetumoruntilthebackmusculatureoftheanimaliscleanlyexposed.

During primary tumor resection, special care must be taken not to remove any of theaxillarylymphnodesthatoftenoccupytheextremelateralbordersofthetumorresectionsite.

ClosetheskinofthemouseusingMichelleskinclips.

The skin should be closed along a line that is perpendicular to the mouse's body axis. Thispreventsimpairmentsinfront-limbmobility.

Inspect and palpate the mice for metastatic disease progression at regular intervalsevery couple of days. Direct attention especially towards the axillary regions andanteriorneckjustundertheanimal'schin.

After tumor is palpable, quantify metastatic burden by measuring bisecting lymphnode dimensions, in square millimeters, at recurrence sites using vernier calipers.Expressdataforeachanimalastheaggregatesumofmetastasis-bearinglymphnodedimensions.

EuthanizetheanimalbyCO_2asphyxiation.Harvestaxillaryandnecklymphnodes,submandibularsalivaryglands,andlungstoassessmetastatictumorburden.

Fixtissuebyparaffinembeddingandsectioning.

Analyze fixed tissue by hematoxylin and eosin staining to confirm thepresenceofTRAMP-C2metastaseswithintheseorgans.

MICRODISSECTIONOFTHEMOUSEPROSTATE

In contrast to the human prostate, which is a single organ that can be histologicallysegregated into several zones (i.e., anterior, peripheral, and transitional), the mouseprostate represents an organ complex composed of four paired-sets of lobes: ventral,dorsal,lateral,andanterior(coagulating).Microsurgicalisolationoftheindividualmurineprostate lobes ensures adequate representation of all four, facilitating valid histologiccomparisonofsimilarglandulartissuesfromdifferentmiceafterexperimentaltreatment.Isolation and recovery of the individual glands of the murine prostate, however, ischallenging given the extremely small size of these glands within the murine prostatecomplex. In fact, experienced urologic surgeons have encountered difficulty with evenlocatingtheprostateinamalemouse.Inadditiontotheirminutesize,theindividuallobesofthemurineprostatearespatial lyseparatedalongtheurethra,intimatelyinvestedwithinfatandconnectivetissues,andcloselyassociatedwithotherpelvico rgansincludingthepairedampullaryductsandseminalvesicles.Consequently,itisimperativetoisolatetheprostatefromthe ampullaryductsandseminalvesiclestoavoidconfusingthehistologyofthesestructureswiththatoftheprostate.Hence,thisp rotocolisintendedtoassistwiththe localization and microdissection of the prostate to facilitate studies in the TRAMPmodel.

Materials

MaleTRAMPmouse(seeBasicProtocol1)70%ethanol
Dissection medium, 4°C: cell culture media (e.g., DMEM) or balanced saltsolution—i.e.,phosphatebufferedsaline(PBS)orHank'sbalancedsaltsolution Smallscissors
Two grade-4 forcepsSmall Vannas scissors
Serratedcurvedforceps
10cmpetriorcellculturedishStereo dissecting microscopeHigh-intensitylamp

Additionalreagentsandequipmentforanesthesiausingmethoxyflurane. Alternatively, tissue can be transiently kept in 4°C DMEMuntiltransferintocassettesormolds.

EuthanizeamaleTRAMPmousebyCO$_2$asphyxiation(*UNIT1.8*)andlayitonitsback.Wettheabdomenwith70%ethanoltoreducecontaminationoftissuesampleswithhair.

Makealargetransverseincisionwithsmallscissors,atleastone-thirdthewayuptheabdomenofthemouse,cuttingthroughtheskinandthemusclewall.

Pullthelowerflapofskintowardthetail.

Theseminalvesiclesandbladderbecomeeasilyvisible.Theprostateislocatedjustbelowthebladderandinold eranimalsissurroundedbyfat

Using grade-4 biological forceps, grasp the bladder and gently scrape the thinconnective tissue attachment connecting it to the body wall and theassociated fat towards the tail until the urethra below the prostate is visible .

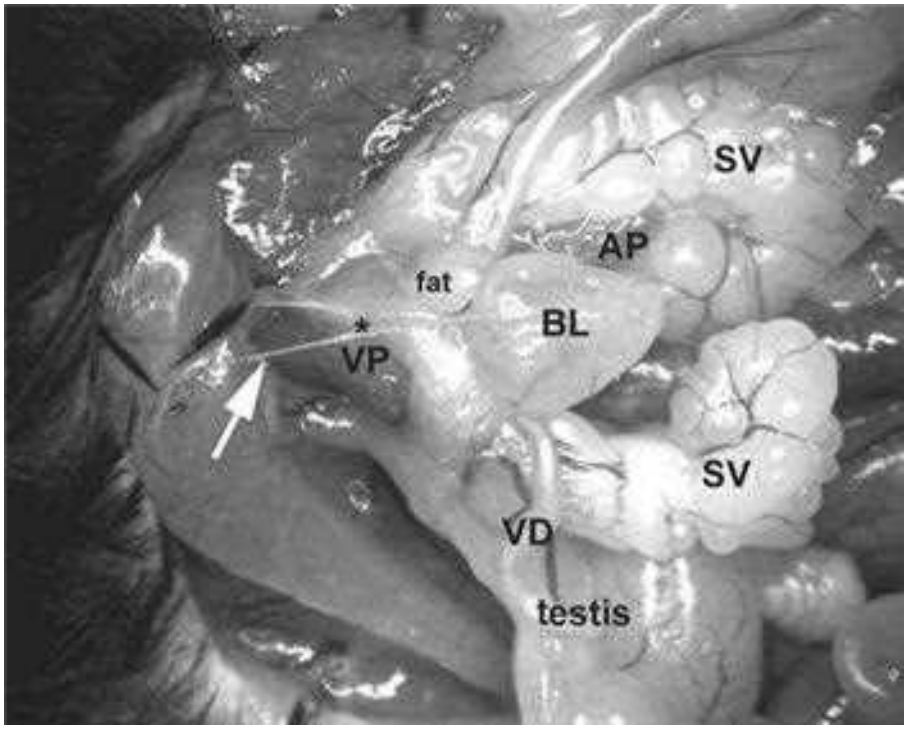


Figure 20.5.1 The reproductive tract in an adult male mouse. The arrow indicateswheretocuttheurethrawhenremovingthereproductivetract.Theasterisk(*)indicatestheconnectivetissuebetweenthebodywallandthebladder.Abbreviations:AP,anteriorprostate; BL, bladder; SV, seminal vesicles; VD, vas (ductus) deferens; VP, ventralprostate.

Usingsmallscissors,cuttheurethra~3mmbelowtheprostateasclosetothepelvicgirdle as possible (see arrow of Fig. 20.5.1), and gently pull the bladder up and outofthebody.

The reproductive tract will come out of the body, but will still be attached by two sets ofducts,theductusdeferensandtheureters.

Using small Vannas scissors, cut each of the ducts and remove the bladder andreproductivetractenblocfromtheanimal.Placetheseorgansintoa10-cmpetriorcell culture dish containing chilled dissection medium under a stereo dissectingmicroscopeilluminatedbyahigh-intensitylamp.

Chilling the dissection medium helps to coagulate the secretions of the accessory sexglands.

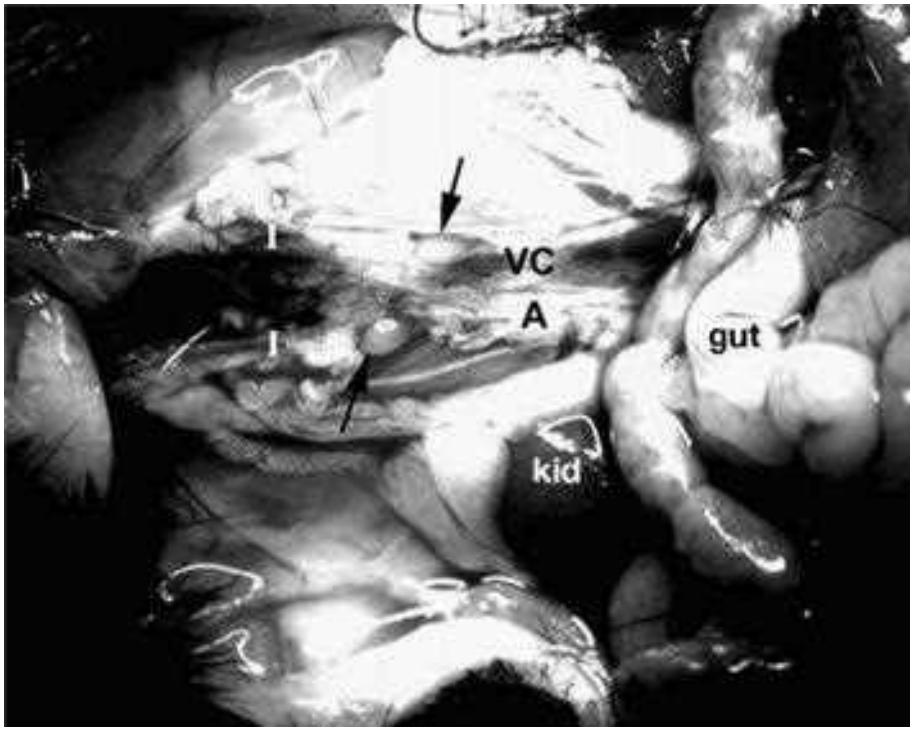
Optional: Remove the lymph nodes, located on either side of the abdominal aortajustaboveitsbifurcationintotherightandleftcommoniliacarteries withserratedcurvedforceps.

The draining lymph nodes of the prostatic complex are located beneath the prostaticcomplex.

Orientthereproductivetractinthedishsothattheventralsideisup.

This is the same orientation as the reproductive tract of a mouse laying on its back. Theseminalvesicleswillbeflatonthebottomofthedishwiththeirtipscurvingtowardseachother.

Duringdissection,therearetwoplaceswherethereproductivetractcanbegraspedwithoutdamagingt heprostate,thebladderandthecutendoftheurethra.Also,avoidperforatingthe seminal vesicles. Fluid from the seminal vesicles is opaque and will interfere withvisibilityduringtherestofthedissection.



Locationofthedraininglymphnodes.Thereproductivetracthasbeenremovedfromanadultmalemouserevealingthedraini nglymphnodes(indicatedbyarrows). They are located on either side of the abdominal aorta above its bifurcationintotheiliacarteries.Abbreviations:A,aorta;I,iliacvessels;kid,kidney;VC,venacava

Next, remove the fat and connective tissue of the reproductive tract to expose theindividuallobesoftheprostate.Todothis,holdthecutendoftheurethra,graspttheconnectivetissuesur roundingtheprostaticlobeswithgrade-4biologicalforceps,andpeelittowardsthetipsoftheseminalvesiclesandawayfromtheprostate.

The major blood vessels are associated with the connective tissue and can be used as avisual guide for removing the connective tissue. The fat pad is delicate and shreds whengrasped;therefore,itisbesttotrytoremovethefattogetherwiththeconnectivetissue.

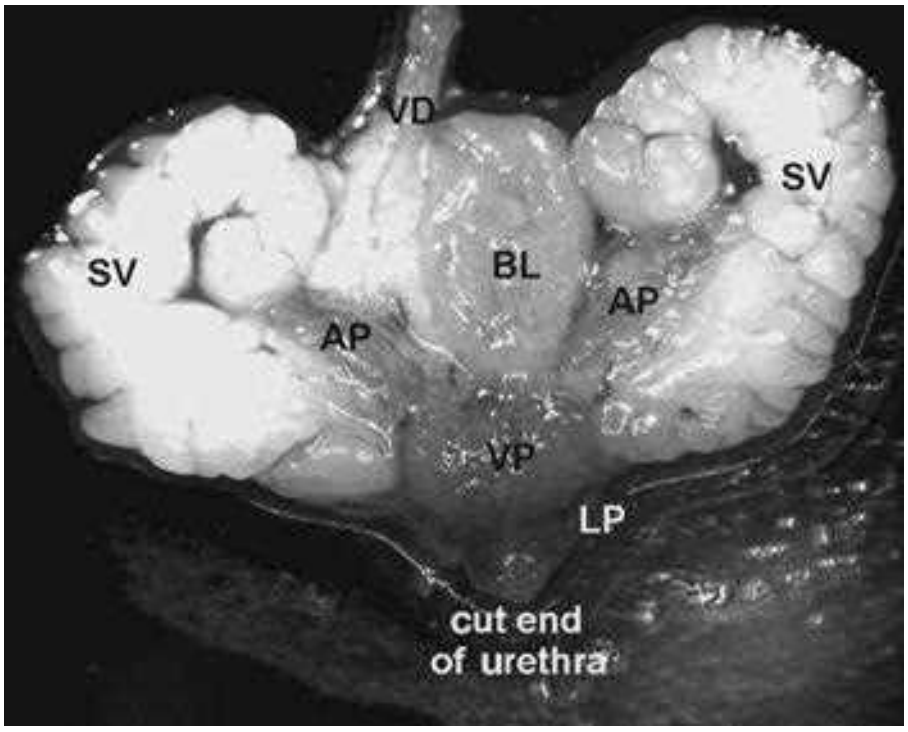
To remove the two lobes of the ventral prostate at their points of insertion in theurethra,visiblebetweenandsuperiortothelaterallobesoftheprostate,holdthecutendoftheurethra,opentheforceps1.5mm,andrunthemupthemidlineof the urethra. Just below the bladder, firmly close the forceps and pull the ventralprostateuptowardsthetipoftheseminalvesicles.

Thepointsofinsertionforalloftheductsfallintoaringjustbelowtheinsertionssitesoftheseminal vesicles.

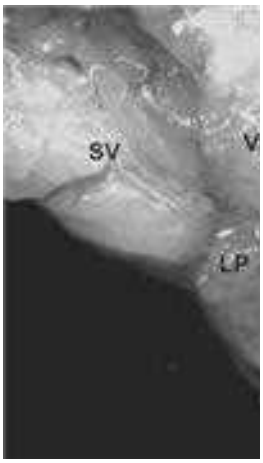
Bothlobesoftheventralprostateshouldberemovedleavingthelateralprostaticlobesstill attached to the urethra. Remove the individual lobes of the prostate at their point ofinsertionintotheurethra.

Ifthelaterallobeisaccidentallyremovedwiththeventralprostate,thelaterallobecanbeidentifiedbyitslaterallocationandmoreopaquesecretions.Also,thelobesofthelateralprostate are smaller than the ventral prostate. To clearly see each individual lobe of theventral prostate prior to its dissection, one may run the point of closed forceps up themidline of the urethra. This will separate the ventral prostate into its left and right lobes.Thepointsofinsertionarevisiblejustbelowtheneckofthebladder.

Withtheventralprostateremoved,thetwosmalllaterallobesoftheprostatebecomeapparentjustlateralto thepointwheretheventralprostateinsertsintotheurethra.



Ventral view of an adult male mouse genitourinary complex. Thiscomplexhasbeenremovedfromanadultmalemouseandplacedindissecting media.Abbreviations: AP, anterior prostate; BL, bladder; LP, lateral prostate; SV, seminalvesicles;VD,vas(ductus)deferens;VP,ventralprostate.


SV
V
LP

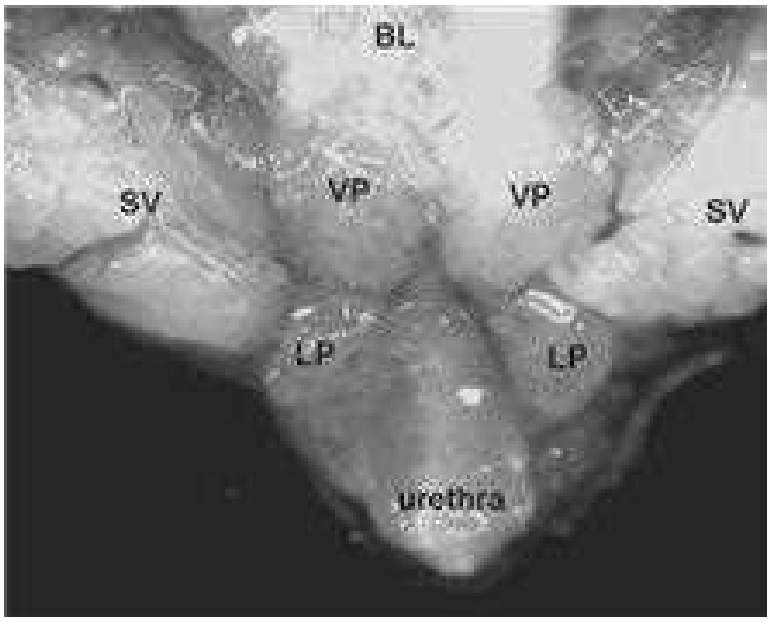
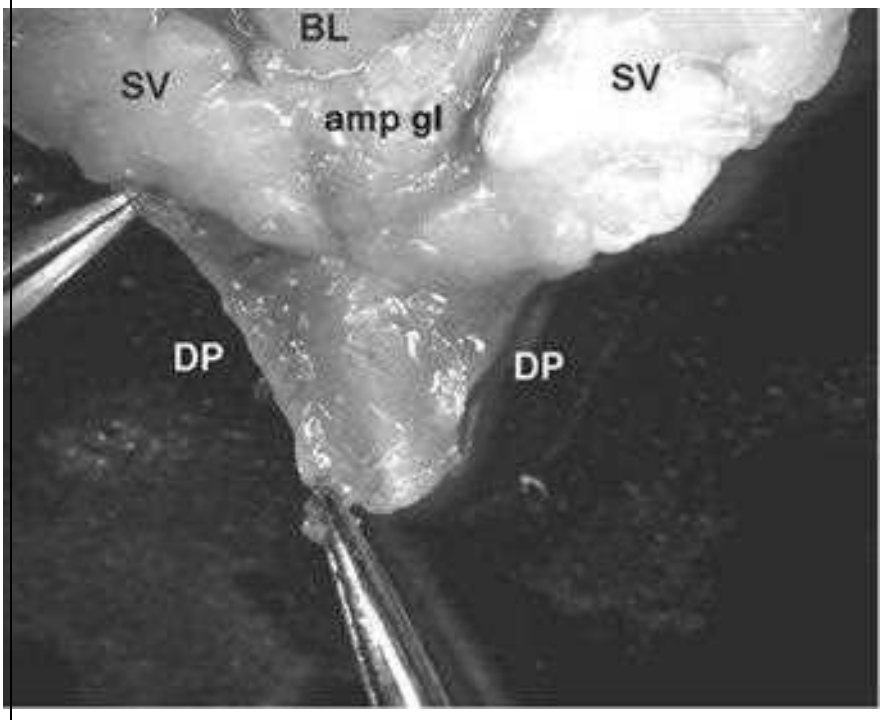


Figure20.5.4 Theventralandlaterallobesoftheprostate.Connectivetissueandfatsurroundingtheventralandlateralprostatehavebeenremoved. Theventralandlaterallobes of the prostate have been surgically separated and their points of insertion arenow visible. Abbreviations: BL, bladder; LP, lateral prostate; SV, seminal vesicles; VP,ventralprostate.



thedorsalviewofanadultmalemouseprostate.Theforcepsdemonstratethelocationofthedorsalprostatejustbelowtheseminalvesiclesontheposterioraspect of the reproductive tract. The ampullary gland is visible between the seminalvesicles.Abbreviations:ampgl,ampullarygland;BL, bladder;DP,dorsal prostate;SV,seminalvesicles.

Remove the lateral prostate using the same technique described in step 10 above.Hold the cut end of the urethra and remove the lateral lobes of the prostate at theirpointofinsertionusinggrade-4biologicalforcepsorVannasscissors.

Continuetoholdthecutendoftheurethra.Removetheanteriorprostatelobes by grasping the two main ducts of the anterior prostate at their base usinggrade-4biologicalforceps.Peeltheseglandstowardsthetipoftheseminalvesicles.

Flipthereproductivetractoversothatthedorsalsideisup.

The dorsal prostate is visible as a pair of triangular glands attached to the urethra justbelow the seminal vesicle . The points of insertion for the seminal vesiclesshouldalsobevisible.

Toremovetheseminalvesicles,holdthecutendoftheurethraandfirmlygraspthebase of the seminal vesicle using grade-4 biological forceps at the point where itbeginstonarrow,~3mmfromtheurethra.Pluckthseminalvesiclesfromtheurethra,takingcaretokeepthforcepstightlyclosedtoavoidfluidfromleakingout.

Theductsofthedorsalprostateextenddowntheurethratowardsthecutend(Figure20.5.5). Remove the thin layer of connective tissue surrounding the dorsal prostateby grasping it at the midline and peeling it up toward the seminal vesicle insertionsite.Detachtheductsofthedorsalprostate,whichextenddowntheurethratowardsthe cut end, by gently running the tip of the forceps under the ducts. Remove thedorsalprostatebycuttingitsductsatthepointsofinsertionorbypluckingthemoffwithforceps.

Removetheampullarygland,whichliesatthebaseoftheductusdeferens(itspointof insertion into the urethra), by cutting at its point of insertion usingsmallVannasscissors.Itisfoundatthecenterofthereproductivetractbetweenthebladderandtheseminalvesicles.

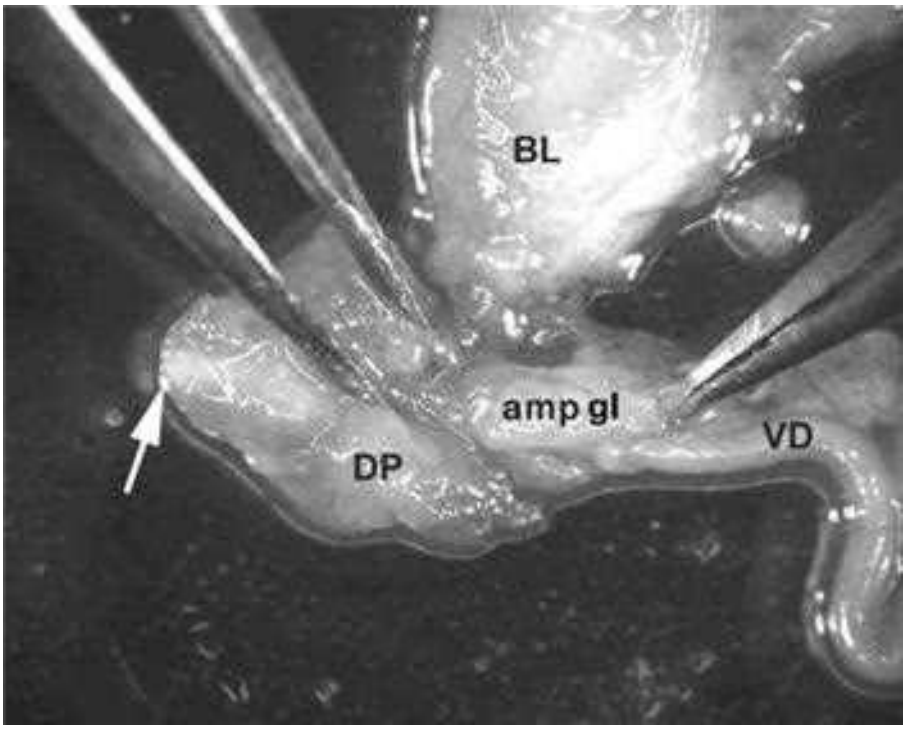


Figure 20.5.6 Dissection of the ampullary gland. The ampullary gland is located atthebaseoftheductusdeferensandmustberemovedwithscissors.Thearrowindicatesthe cut end of the urethra. Abbreviations: amp gl, ampullary gland; BL, bladder; DP,dorsalprostate;VD,vasdeferens

Table20.5.1 HistopathologicGradingofTRAMPTumors

Histopathic score[a]	Histopathicclassification	Description
1	Normalprostate	Inthenormalprostate,epithelialcellscomprisingtheglandsare uniforminsizeandorientation,andtheirnucleiares mallandwell-defined.
2	Early intraepithelial neoplasia	
3	Advanced intraepithelialneoplasia	
4	Well-differentiated prostateadenocarcinoma	

5	Moderately differentiatedprostateadenocarcinoma	Early neoplastic changes are evident as an increase in epithelialnuclear-to-cytoplasmic ratio and “tufting up” of the epithelial layer orlayersintotheglandularlumen.Thislesionisanalogoustoalowgrade PIN lesion in humans.
6	Poorly differentiatedadenocarcinoma	Thislesionisanalogoustoamore-advancedPINlesioninhumansandis associated with extensive infolding of epithelial cell layers into thelumenandanincreaseinbothmitoticand/orapoptoticfigures. Thisgradeofcancerisrepresentedbyearlyinvasion/penetrationoftheglandular basement membrane by tumor cells that extend into thestromalcompartment Moderately-differentiatedcancerisrepresentedbytumorformationofprimitive glands lacking an obvious lumen. Tumor cells comprisingtheseglandshavelosttheirtallsecretoryappearance. This most severe grade of prostate cancer is represented by tumorscomposedofsheetsandcordsofhighlypleiomorphicanaplastictumorcells.

[a]Tumorgradesoflessthan4areinfrequentlyassociatedwithmetastaticlesions.Ifhistopathologicclassificationisevenlydividedbetweentwoscores,thenadecimalscorecanbeassignedinincrementsof0.5.

SCORINGTUMORSINTHEAUTOCHTHONOUSTRAMPMOUSE

Four separate parameters can be assessed and used, either alone or together, to scoretumorsinTRAMPmice.Theseincludegenitourinary(GU)weigh,primary tumor incidence , metastases, andtumor grade . Prior to microdissection andprocessing of tissues, the en bloc GU complex (including the prostate lobes, seminalvesicles, and drained bladder) can be weighed as one index of primary tumor burden inthese mice . Alternatively, to determine primarytumor incidence, one can record the presence or absence of tumors, first appearing aswhitishnodulesemanatingfromoneorseverallobesoftheprostateduringmicroscopicdissection. This procedure is relatively simple and particularly useful when treated anduntreated TRAMP mice are being compared at ~16 to 18 weeks of age—i.e., whenestablishedbutrelativelysmall(2to10mm)tumorsarecommonlypresentinthenormalTRAMP mouse. Primary tumors should be confirmed by histopathologic examination.Thescoringofmetastasesisslightlymoredemandingandrequiressamplingandformalhistologicevaluationo forganscommonlyinvolvedbymetastatictumorspread,includingdraining retroperitoneal lymph nodes (i.e., periaortic, pericaval, and renal hilar lymphnodes),lungs,kidneys,andliver.Finally,togradeprostatecancersinTRAMPmice,theindividual lobes of the prostate should be immediately fixed overnight in 10% neutral-buffered formalin (or 4% paraformaldehyde at 4°C for 4 hr). For convenience, a nine-chambercassettecanbeusedtoembedalltissuesfromoneanimalinparaffin.Paraffin-embeddedtissuescanthenbesectionedandstainedwithhematoxylinandeosinusingstandardmethods.

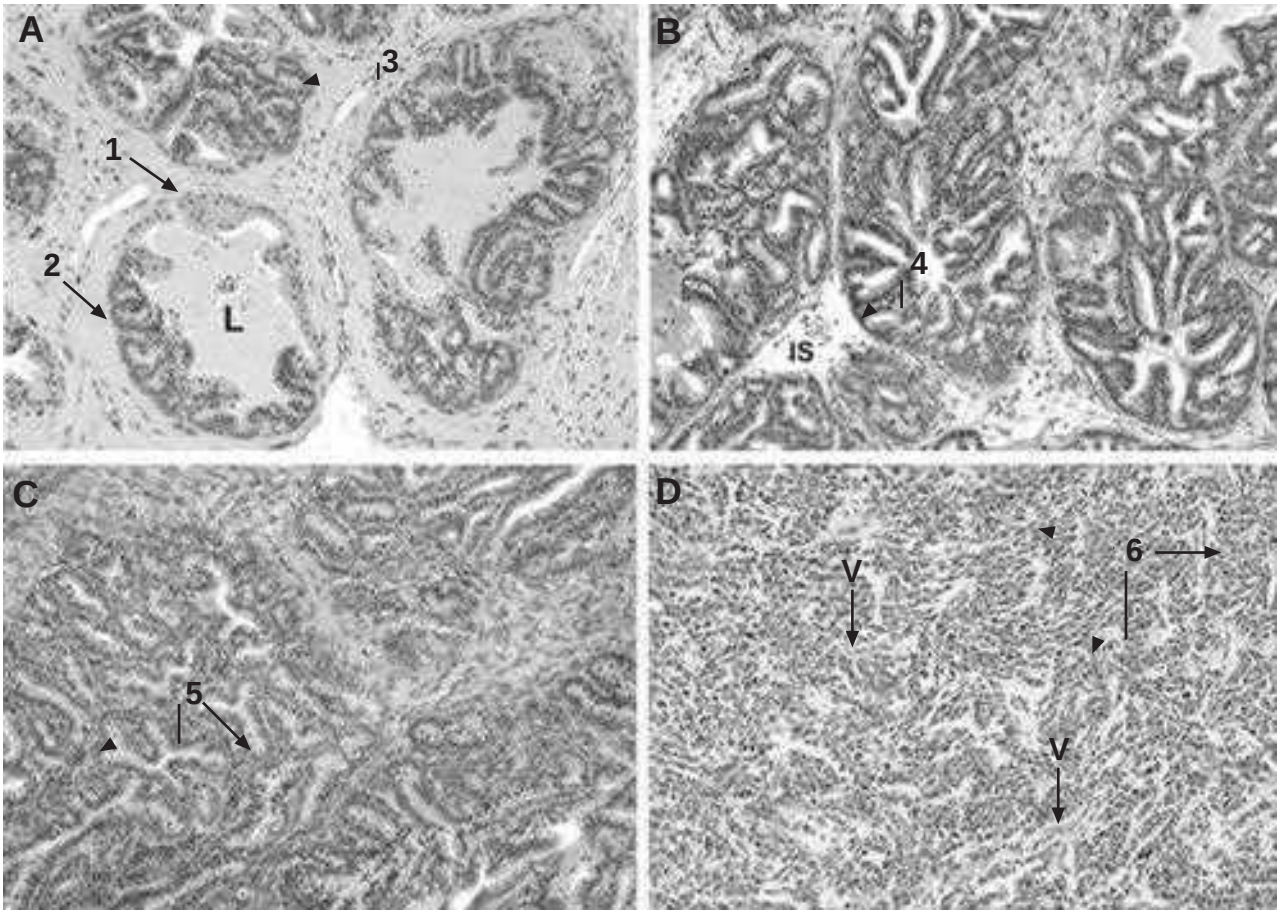


Figure 20.5.7 Histologic grades of TRAMP tumors. TRAMP tumors were microdissected and prepared for routinehistopathologic analysis. The numbers indicate the score corresponding to the histologic grade of the lesion depicted byeacharrow.Asshowninpanel(**A**),earlyprostaticlesionscanconsistofavarietyofhistologicgrades,rangingfromnormalepithelium(1)tolowgradePIN(2),ormoreadvancedPIN(3).Thelumen(L)ofasingleacinarstructureisindicated.Moreadvanced lesions are detected in older mice. In panel (**B**), the initial stages of adenocarcinoma (4) are represented by anarrowing or loss of interductal spaces (IS) and substantial reduction in the size of acinar lumens. In panel (**C**), moreadvancedadenocarcinoma(5)ischaracterizedbylossoflumenalandductalstructuresandaless-differentiatedphenotype. Finally,panel(**D**)depictsasolid,well-vascularized(V)tumorcomprisedofsheetsofanaplastictumorcells(6).

Prostatetissuesareevaluatedbasedonthedegreeofneoplastictransformation.Wehavepreviously reported a grading system (Hurwitz et al., 2000; Gingrich et al., 1999)analogoustotheGleasonsystemwhichisusedtoscorehumanprostatecancer(Gleason,1977).Ino ursystem,prostatetissuesareassignedanumericalscorebasedonthecriteriadescribedinTable20.5.1(al soseeFig.20.5.7).Thesignificanceoftumorgradeisbornout of the tendency for prostate cancers to become more poorly differentiated as theyprogress. From this, it follows that potentially promising experimental therapies mightmanifestasanabilitytoreduceorarrestthisprogressionofprostatetumorgrade,orevenreverse this process by inducing a more differentiated phenotype. Hence, the followingprotocolprovidesausefulandestablishedguideforthegradingofautochthonoustumorsinthe TRAMPmodel.

Materials

Microdissectedprostatelobes
Additionalreagentsandequipmentforparaffinembeddingandsectioning,andhematoxylinandeosinstaining

Embed microdissected prostate lobes in paraffin, section (*UNIT 5.8*), and stain withhematoxylinandeosin.Examinemicroscopically.

Alternatively, to facilitate immunohistochemical analysis, cryopreserved tissues can beevaluated . Examination of cryosections, however, should not be attempted forgrading TRAMP tumors; therefore, it is often helpful to snap-freeze one lobe and fix thecontralateralloberforparaffin-embedding.

Determine the mean overall score (Table 20.5.1) after examining all four prostaticlobesofeachanimal.

Togenerateascoreforanindividualmouse,thescorecorrespondingtothepredominanttumor grade is determined blindly. A mean value for animals within a given cohort canthen be calculated by averaging the individual scores of animals within that treatmentgroup.

CASTRATIONOFMICE

As occurs in man, androgen withdrawal by castration in the autochthonous TRAMPmodelresultsininvolutionofprostatetissuesaswellastheseminalvesicles.Despitethisinitialresponse,whichpre sumablyalsoinvolvesregressionofandrogen-dependenttumorcellsandperhapsattendantdown-regulationofandrogen-inducedneoplastictransforma-tiondrivenbyTAgexpression,upto80%ofTRAMPmicecastratedat12weeksofagewillexperiencesubsequentprostatet umorrecurrenceanddiseaseprogression. This phenomenon parallels the emergence of “androgen-independent”diseaseasoccursinpatientsfollowingthefailureofstandardhormone-ab-lativetherapyeitherbysurgicaland/orpharmacologiccastration.Itisthisformofprostatecancer that is thought to be responsible for the vast majority of deaths for patients withadvancedprostatecancer.Inlightoftheimportantroleofandrogensinthepathogenesisand treatment of prostate

cancer, and the need for relevant murine models that mimic“androgen-independent” disease progression, the following protocol is provided forandrogenablationinthemurinemodelviacastration .

Materials

12-week-oldmaleTRAMPmice(seeBasicProtocol1)10%povidone-iodine
Irisscissors
Electrocauterydevice(Roboz)2smallhemostats(optional)
Michellesurgicalclips(Response)ornonabsorbablesuture

Additionalreagentsandequipmentforanesthesiausingmethoxyflurane(*UNIT1.4*)

Anesthetize a 12-week-old male TRAMP mouse with 15% isoflurane (using tech-niquesasformethoxyflurane;*UNIT1.4*).Cleanthescrotumandperineumofthemousewith10%povidone-iodine.

Withthemouseonitsback,pinchitsabdomenfirmlybetweenthumbandindexfingertoexpressthetesticlesintothescrotum.

Useapairofirisscissorstomakeasmall(3-mm)transversecutacrossthescrotumand underlying tissues until the shiny gray-white surface of the testicles is clearlyapparent.

Pulleachtesticlethroughthescrotalincision(oneatatime)andtransectthespermaticcord, as close to the testicle as possible, using an electrocautery device to providehemostasis.

Alternatively, the spermatic cords can be stretched and snapped between two hemostatswithminimalbloodloss.
Pushthespermaticcordstumpsbackintothescrotumandclipthescrotumclosedwithasingle Michellesurgicalcliporasinglenonabsorbablesutur .

Extremecaremustbetakentoavoidinjuryorobstructionoftheurethra,penisand/oranusofthemouse,especiallywhenthesurgicalclipisappliedfollowingcastration.

REAGENTSANDSOLUTIONS

Usedeionized,distilledwaterinallrecipesandprotocolsteps.Forcommonstocksolutions,see

Oligonucleotideprimerstocks,10***μ***Meach

MousebCasein(MbC)forwardprimer:
5¢-GATGTGCTCCAGGCTAAAGTT-3¢
MbCreverseprimer:
5¢-AGAAACGGAATGTTGTGGAGT-3¢
Probasin(PB)-1forwardprimer:
5¢-CCGGTCGACCGGAAGCTTCCACAAGTGCATTTA-3¢
T-antigen(TAg)reverseprimer:
5¢-CTCCTTTCAAGACCTAGAAGGTCCA-3¢

MbCfunctionsasaninternalcontrol.

PCRmastermix

5ml10´*Taq*bufferwithoutMg^{2+}
1ml10mMdNTPmix(Gibco/BRL)6ml25mM$MgCl_2$
1.25mleach10mMoligonucleotideprimerstock
0.5ml*Taq*polymerase
31.5ml distilledH_2O
Makefreshanduseimmediately

*Thetotalvolumeis49*µ*l.*

Taillysisbuffer

10ml1MTris×Cl,pH8.5

4ml5MNaCl(*APPENDIX2A*)

1ml0.5MEDTA(*APPENDIX2A*)

1 ml10%(w/v)SDS(*APPENDIX2A*)

Adjustvolumeto100mlwithdistilled,deionizedH_2OStoreupto1monthatroomte
mperature

TRAMPtissueculturemedium

Dulbecco'sModifiedEagleMedium5%(w/v)FBS
5%characterizedfetalcalfNuSerum(CollaborativeBiomedical)5mg/mlinsulin,cell-culturegrade
0.01nMdihydrotestosterone(Sigma)
100U/mlpenicillin/100mg/mlstreptomycin .

AnticipatedResults

TAg is first expressed within the prostate ofTRAMPmiceatpubertywhichis~3to4weeksof age . By 12 weeksofage,maleTRAMPmiceexhibittheearliesthistologic evidence of disease discernible asprostaticintraepithelialneoplasia(PIN).By18to20weeks,frankcancersaretypicallyappar-ent predominantly in the dorsal and laterallobes of the prostate.
By28to32weeks100%ofTRAMPmicewillhaveadvanceddisease,encompassingmetastasestothe pelvic and retroperitoneal lymph nodes inproximitytothegreatvesselscaudaltotherenalhila . As occurs in humans,tumorprogressionintheTRAMPmodelcan also result in metastases to the viscera,including the lung (~40% of mice by 30 weeksofage),kidney,andoccasionallytheboneandmarrow. While some macrometastases can beevidentwithinthelung,mostmetastasestothelung require microscopic visualization. Visualizationofmicrometastasestothelymphnodes is greatly facilitated by immunohistochemicalstainingoftumorcellsusingananti-pancytokeratin antibo. Extensive local tumor invasionoftenproducesprofoundseminalvesicledistention in the pure C57BL/6 TRAMP modelandalsocanresultinbladder-neckand/orureteral obstruction resulting in hydronephrosisandkidneyfailure.Veryinfrequently(<1in 100 mice) extraprostatic tumors may presentaseitherwidespreadmetastasesorbulkynodaldiseaseintheabsenceofadiscernibleprimaryprostate lesion. Even less frequently (<1 in 500mice), we have observed female TRAMP micedevelopingpoorlydifferentiatedanteriornecktumors. At present, data pertaining to rates ofsurvival of TRAMP

mice are still relativelylimited. Based on “survival” studies, we haveobservedthat~50%ofheterozygousC57BL/6TRAMP mice were alive at 33 weeks of age,with the longest survivors living to 48 weeks.This estimate of survival is in rough agreementwithothervaluesreportedintheliterature.

For studies pertaining to androgen-inde-pendentprostatecancerprogression,followingcastration of 12 week-old TRAMP mice, onecan expect up to 70% to 80% of these mice todeveloprecurrentandprogressivetumorsafteran initial interval of genitourinary (GU) com-plexinvolution .Involu-tion of the GU complex is evidenced by areduction in the combined weight of the blad-der,prostate,andseminalvesiclesaftercastra-tion.ReductionsinGUweightareappreciableas early as 2 weeks (and likely much earlier),and can persist for up to 12 weeks followingcastration. Based on data obtained 12 weeksfollowing castration, androgen ablation doesnot ultimately delay or reduce prostate tumorandmetastasisformationinTRAMPmic. Cancer progression inthecastratedTRAMPmouse,however,isstrongly associated with an increase in the se-verity of tumor grade. In one study, 100% ofTRAMPmicecastratedat12weeksofagewereshown to harbor poorly differentiated cancersby 18 and 24 weeks of age, compared to only27% of control (androgen-intact) mice evalu-ated at similar time points . Hence, the castrated TRAMP mouseprovides a relevant model for studying andro-gen-independent disease progression and fur-ther suggests that androgen-independent dis-easemaybeassociatedwiththeemergenceofapoorly-differentiatedphenotypeinthismodel.

With regards to breeding, it is important torememberthatifonecrossesheterozygousTRAMPmicewitheithernormalC57BL/6orFVB/Nmice,onecanexpect50% oftheprog-eny to be positive for the transgene; however,sinceonlyhalfofthesetransgenicmicewillbemale(25%ofthetotallitter),breedingmustbeplannedac cordingly.Finally,wehavefoundthatbreedingTRAMPmicewithintheC57BL/6backgroundyieldsrelati velysmalllitters(~4to6mice/litter),whileTRAMPF_1litters are generally larger (~8 to 12 mice/litter).FortheSubcutaneousTRAMPtumor mod-els (see Basic Protocol 2 and Alternate Proto-col),ratesoftumoroutgrowthfrominjectedTRAMP-Ccellsarerelativelyslowcomparedtootherestablishedtumorcelllines.Followinginoculationwith2.5–5.0´10^6TRAMP-Ccells,log-phasetumorgrowthtypicallybe-comes apparent 2 to 3 weeks after subcutaneoustumorcellinjectio.Thesetumorsgenerallycanachieve250mm^2dimen-sionswithin8weeksandarecommonlyasso-ciatedwithreddeningorulcerationoftheover-lyingskin. Early on,thesetumors aredenselyadherenttotheoverlyingskinbutcanbecomeinvasiveintothemuscleandevenbonewithprolonge dgrowth.AlthoughbothTRAMP-C1and-C2expressandrogenreceptor,wehavenot

observed classical hormone-mediated tumorregressionfollowingcastrationofmicebearingestablished TRAMP-C tumors; therefore, thequestion as to whether these lines are andro-gen-dependent remains unresolved. With re-gards to the TRAMP-C2 tumor resection/me-tastasismodel,surgeryisgenerallywelltoler-ated by the animal with a mortality rate of <1%.Overt metastatic tumors become apparent be-tween 20 to 60 days following primary tumorresection . Greater than 95%of these mice demonstrate metastatic failurefollowing primary tumor resection. Diseaseburdeninthismodelcanbequantifiedlongitu-dinally by measuring TRAMP-C2 metastaticoutgrowthintheaxillaryandanteriorcervicallymph nodes. We have previously shown thatmetastatic failure in this model occurs conse-quenttoprogressivegrowthofestablishedTRAMP-C2 micrometastases present

withinthese draining lymph nodes at the time of pri-mary tumor resection . Incontrast,localtumorrecurrencesoccurin

£10% of mice, and local disease volume isgenerallysmallcomparedtothatatmetastaticsites. Finally, micrometastases can be foundwithin the lungs and submandibular salivaryglandsin~50%ofmicethatexperiencemetas-taticfailurefollowingprimarytumorresection(i.e.,whenaggregatemetastatictumordimen-sionachieves250mm^2).

Time Considerations

For breeding TRAMP mice, one must allowforthenormalintervalsof19daysforgestation,3 weeks until weaning, and ~6 to 8 weeks untilasecondroundofbreeding.SpecifictoTRAMP mice are the following intervals: 12weeks until the development of prostate in-traepithelial neoplasia (PIN), 18 to 20 weeksuntil macroscopic prostate tumor formation,and28to30weeksuntilestablishmentofme-tastases.ForscreeningTRAMPmice,oneshould allow 2 to 4 days for tail DNA extrac-tion,PCRtransgeneamplification,and gelanalysis. For experiments pertaining to prostatemicrodissectionandtumorrecovery,allow~30min for each dissection. Tissue can then beprocessed immediately (by snap freezing inliquid nitrogen) or fixed overnight for perma-nent sectioning. For castration experiments,permit5minpermousesurgery.Forsubcuta-neoustumorexperiments,permit2to3weeksforculturingTRAMP-Ccells(forlargeexperi-ments requiring 10×10^7 cells), 1 hr for har-vestingandpreparingTRAMP-Ccellsuspen-sions,and~1hrforinjecting15to30mice.Inthe subcutaneous models, first tumor growthshould be apparent by 2 to 3 weeks, and by 6to 8 weeks most tumors will have achieved250-mm^{2}dimensions assuming an initial in-oculationof$2.5–5.0 \times 10^6$cellspermouse.Forthe subcutaneous tumor resection/metastasismodel,permit~15minpermousetumorresec-tion. Metastatic tumor recurrences occur be-tween 3 to 8 weeks following primary tumorremoval, and most animals achieve an aggre-gate metastatic tumor dimension of 250 mm^{2}by9to16weeksfollowingtumorresection.

ANIMALHEALTHMONIORINGINICMR NARFBR

DEPARTMENTOFBIOSCIENCE&BIOTECHNOLOGY

Fortheawardofthedegreeof

MASTEROFSCIENCE
IN
BIOTECHNOLOGY

Submittedby

S.JESSEJOY

MSc (BIOTECHNOLOGY)

UndertheGuidanceof:

Dr. L.SUSEELA

HEADOFTHE DEPARTMENT

DEPARTMENTOFBIOSCIENCE&BIOTECHNOLOGY

KRISHNA

UNIVERSITYMACHILIPATNA

M-521001, A.PJULY/AUGUST-

2023

DECLARATION

I hereby, declare that the subject matter embodied in this project report ,entitled **"ANIMAL HEALTH MONITORING IN ICMR NARFBR ''.**Which being submitted by me, for degree of the Master of Science in Bio Technology. Krishna University Machilipatnam,(A.P),India,istheresultofresearchbymeunderthe guidance of **Dr.L.SUSEELA**HEADOFTHEDEPARMENT,Department of Bio Science & Bio Technology . KrishnaUniversity,Machilipatnam

Place:Machilipatnam

Date:

(S.JESSE JOY)

CERTIFICATE

This is to certify that the project report entitled "ANIMAL HEALTH MONITORIN G IN ICMR NARFBR" is a bonafied work carried out by Ms. S. JESSE JOY (Y21BIT101021) under my supervision is submitted in partial fulfillment of the requirements for the award of degree of Master of Science Bio Science & Bio Technology to Krishna University Machilipatnam, A.P. No part of the dissertation has been submitted for any degree/diploma or any other academic award any where before.

PLACE: MACHILIPATNAM
DATE : 18/9/23

Dr. L.SUSEELA 18/9/23

(PROJECT ADVISOR)

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EXTERNAL EXAMINER

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when I present this project report on the completionOfmyresearchwork,Irememberwith gratitude the source of inspiration. Materials , assistance and guidance that lead to this achievement of goal.

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S.JESSE JOY

AnimalHealthMonitoring–ICMR-NARFBR.

ABSTRACT

Laboratory animal experimentation in bio-medical research continue to remain crucial to find out better ways to understand , prevent, treat and cure diseases as currently there are no existing alternatives to substitute the biological systems. Worldwide, new drug research as well as tests meant for assuring the quality, safety, and efficacy of pharmaceutical products/vaccines/recombinant products involves experiments using animals. The use of animals for research is essential for the development of new and more effective methods for diagnosis and treatment of diseases that affect both human and animals.

Pathogens present in the environment are the biggest source of diseases and epidemics in the breeding and experimentation of laboratory animals. The ICMR NARFBR offers preclinical testing on laboratory animals (rodents), large animals (Sheep, Goat, pigs and Horse) and Non Human Primates under one roof.In fact, presence of microorganisms can critically influence the animal health status and reproducibilityofexperimentaldata.TheICLASandWHOguidelinesareformulated with aim of guarantee the best animal health state and valid support for research studies. In this preliminary study, health- monitoring program was carried out within the breeding of laboratory animals in ICMR - NARFBR facility.

The hygienic standards of ICMR have been developed over the last 100+ years. The key element of hygienic standardization is the monitoring of infectious agents that can compromise the animal's health, ordangerous for the personnel or interfere with the research. The health monitoring of laboratory rodents is essential for ensuring animal health and standardization in biomedical research. Progress in **Housing,Barriersystem,zoonosispreventionandhygienicmonitoringprograms** ledto enormous improvement of the microbiological quality of laboratory animals. The main viruses were analysed through molecular diagnostic techniques (PCR, RT-PCR).Bacteria were analysed through (STREAK PLATE METHODS, STAINING METHODS) and Enzyme immunoassays (ELISA-indirect). The established surveillance programme steadily guarantees animal health and ensure the most controlled

environmental and sanitary conditions.Further animal based research plays key role in pre-clinical studies and regulatory research in bio-tech, bio-pharmaceuticals and biomedical institutions across the country.

Keywords:

Healthmonitoring,Hygienicstandardization,Moleculardiagnostics.Rodent Pathogens. Researchvalidity,Micro biome analysis.

INTRODUCTION

National Animal Resource Facility for Biomedical Research (NARFBR). The state government of Telanganaoffered 100 acres of land free of cost in the Genome valley,abiotechhubinTelangana.TheIndianCouncilofMedicalResearch(ICMR), DepartmentofHealthResearch,MinistryofHealthandFamilyWelfare,Government of India has established National Animal Resource Facility for Biomedical Research (NARFBR) at the Genome Valley, Hyderabad .

ThecabinetcommitteeheadedbytheHonourablePrimeMinistergavetheapproval for the formation of NARFBR on 18th November 2015. The Dept. Of Health Research, MinistryofHealthandFamilyWelfareconveyedtheorderson3rdDecember,2015 and subsequently, Indian Council of Medical Research(ICMR) issued a notification on 1st January, 2016 for the creation of NARFBRasa permanentinstituteunderthe aegis of ICMR.

ICMR- NARFBR has been established with the Vision to establish a state-of-the-art infrastructural facility for pre-clinical animal Experimentation of basic, applied, and regulatory research in the country. The institute offers preclinical testing on laboratory animal (rodents), large animals (Sheep, Goat, pigs and Horse) and Non HumanPrimates under oneroof.Indiaisreadytoserveas per'OneHealth Approach' and wholeheartedly committed for human welfare through Science as wellas honoursthe Constitutionalan moral obligations in order toensure animal welfare.

In ICMR NARFBR when working with laboratory animals (mostly**RodentBreeding**facility), scientists need to ensure that animals are not suffering from naturalinfections.Therefore,hygienicstandardshavebeendevelopedoverthe last100+

years. The key element of hygienic standardization is the monitoring of infectious agents that can compromise the animal's health, are dangerous for the personnel or interfere with the research. However, scientists became aware that by eliminating such unwanted infectious agents the overall diversity of all microbes in research animals, the so-called**micro-biome**, has also been reduced. Moreover, it became clear that the micro-biome composition has an enormous impact on how research models react, e.g., to treatments. This might hinder the translation of findings in preclinical research to the clinical situation.

The health monitoring of laboratory rodents is essential for ensuring animal health and standardization in biomedical research. Progress in**Housing, Barrier system, zoonosis prevention and hygienic monitoring programs**led to enormous improvement of the microbiological quality of laboratory animals. While traditional health monitoring and pathogen detection methods still serve as powerful tools for the diagnostics of common animals.

REVIEWOFLITERATURE

As the most common research mammal in the world, mice are key to many aspects of biomedical research and in turn, scientific and medical advancements. Without mice,wewouldlackacrucialunderstandingofmajordiseases,including**cancerand genetic disorders like muscular dystrophy**, which has led to lasting benefits for humans and animals alike. It was only due to studies in mice and other animals. Forexample,wewereabletodevelopvaccinesforCovid-19,andatsucharapidrate.

Byusingmice,researcherscanstudyhow thesediseasesworkinalivingorganism, and importantly, in one that is a good match for humans in many aspects. These principles then underpin the development and testing of new drugs, therapies and interventions. It is therefore not surprising to find that mice are the most used animal for scientific purposes in the EU, making up more than half (52.5%) of the total number of animals used in research – 5,459,433 animals in 2019.

• Few mammals have been as closely studied as the mouse.In this page we will explore some of the research areas in which mice have played a central role, as well as the discoveries and breakthroughs that have come about as a result, and theefforts in other countries to reduce, replace and refine their use**.**

Whyaremicesoimportantinbiomedicalresearch?

Therearesevera limportantreasonswhymicearesowidelyusedin animalstudies for biomedical research:

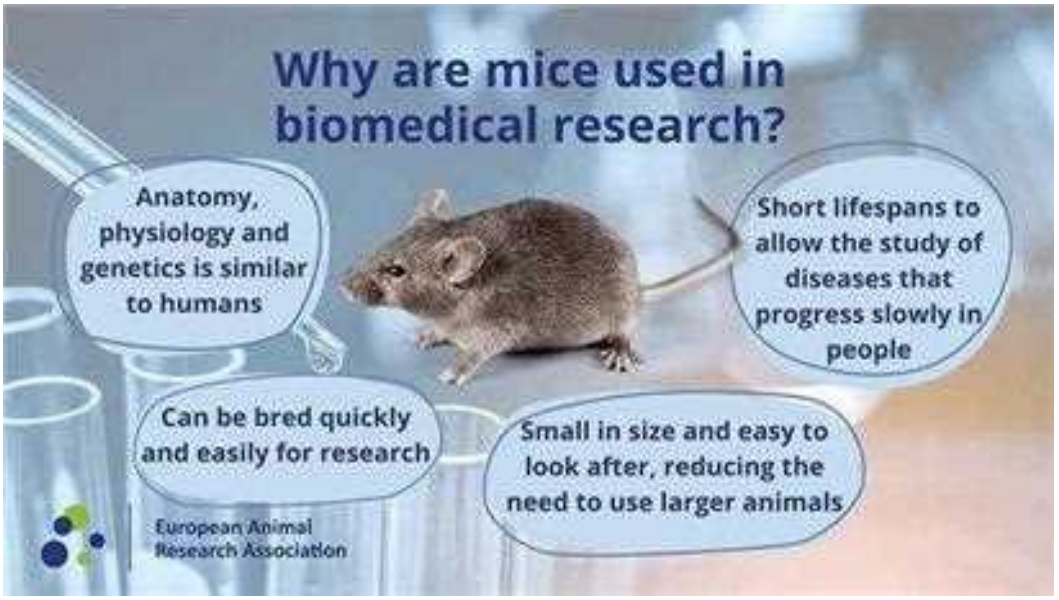
• Miceexperiencemanyofthe**samediseasesashumans**andhavethesametypes oforgansandbodilysystems,whichmakesthemanditsgeneticmap(genome) hasbeen fully sequenced, which means that its genes can be switched on and off to study their effects.

• Mice have short lifespan (2-3 years), making them ideal for looking at **the progression of diseases** thatmay otherwise take years to develop and study inhumans.

• Miceare**quickandeasytobreed**,andweanlargelitters,withagestationperiodof around three weeks. This means that sufficient numbers of mice needed forresearch are available, which doesn't slow the pace of research – something particularlyimportantforstudyingtheeffectsofageing,andinvaccinedevelopment for a new, or rapidly developing disease.

• Fruitfliesandzebrafishareincreasinglyusedinresearch,butifneitherisasuitable model, the mouse is often the **first type of mammal** considered instead.

•Some scientists consider the differences between wild and laboratory mice to be so great that they think these laboratory animals could be classified as a **separate species**.



Whichareasofbiomedicalresearchusemice?

Infectious diseases

Micehavebeencentraltothestudyofmanyinfectiousdiseases,including**influenza, hepatitis and Ebola** – all of which have the potential to be life-threatening. Where mice can be especially useful is in assessing how prone people are to certain infections and investigating why some do not develop immunity. While researchers can investigate these questions in human cells in the lab, these studies cannot

modelthecomplexinteractionsthattakeplaceduringaninfectionasaccuratelyas in a living organism.

Researchers do conduct studies on humans, but not to the extent that is possible in mice.For instance,micecanbefedidenticalandtightlycontrolleddiets,orbeinbred to target the effect of different procedures on the same individuals – things that would be considered unacceptable using humans. It would also be too dangerous to test compounds and drugs on people without first knowing what the possible effects (andrisks)wouldbe,soanimals suchas mice areneededtofulfilthis **crucialrolein toxicity and safety testing**, in everything from experimental cancer drugs to vaccines.

FightingCovid-19–thevitalroleofmice

Covid-19vaccines weredevelopedatexceptionalspeedand studies using mice werevital in this process. Even though mice cannot catch Covid naturally, they can be infectedwiththeSARS-CoV-2virus,bycreatinggeneticallyaltered**'humanised' mice** (see box) that can then be used to examine the disease in detail. The genomes ofthesemice arealteredso thattheyhave a receptorfortheACE2protein,found inhumans, that allows SARS-Cov-2 to enter and infect cells.

Years before the Covid-19 pandemic, mice had already been used to gain important insights about other related diseases, such as SARS, which caused deadly widespreadoutbreaksintheearly2000s,andMERS,whichfirstemergedin2012.It wasthankstotheseearlierstudiesthatresearcherscouldstudyhowthe SARS-CoV- 2 virus infected human cells, without needing to start from scratch with a new mouse model that would have inevitably slowed the pace of Covid-19 research.

Forexample,inresearchattheUniversityofIowa,USA,backin2007,scientistsused humanised mice expressing ACE2 to identify a path to lethal infection by the coronavirus that causes SARS, helping to understand how the disease develops to devise new treatments.

HumanisedmiceforCovid-19allowresearcherstoanswer**keyquestionsabout the virus** itself, such as how it begins to infect organisms and is transmitted from one body to another, how an infected host's immune response reacts, as well as the short- and long-term effect of various treatments and experimental vaccines. All of these aspects are needed to shed light on how the disease worksin order to develop effective drugs and measures against it.

Credit:UniversityMedicineatJohannesGutenbergUniversityofMainz

HUMANISED MICE

Miceandhumansare geneticallyverysimilar,withalmostallmousegenessharing the same functions as our genes. But there are still limits to how much we can compareourselvestomice,especiallywhenitcomes toresearch.Thatiswhylarge numbersofmiceusedinbiomedicalresearcharegeneticallyaltered(GA)to**mirror more human-liketraits** andbiology,typicallyatthe levelofcells or genes.

This‘humanising’processisdonebyinsertingsomethinglikeafragmentofhuman DNA or a tumour into them, sothat the mice react ina similar way to a human.Humanisedmicethereforeallowresearcherstoexplorefarmorehuman genes, **proteins and processes** than would otherwise be possible.

BecauseofthecurrenttechnicallimitationsofgeneediTing,noteveryGAanimalthat is bred for this purpose can be guaranteed to display the genetic mutations or characteristicsthatwereintendedforaparticulartypeofresearch.Itmeans therefore that they are bred but not usedin research. However, the actual breeding ofa GA mouse is counted as a research procedure and is recorded – these mice came toa total of around 12.5 million animals in 2017 (when they were last recorded).

The ’bred but not used’ process is currently being refined through the use of powerfulandaccurategeneeditingtools,suchasCRISPR,thatcandirectlyedit mouse embryos to reduce the number of animals that are needed. You can read moreabout the numerous diseases that CRISPR mice have used to study in this Review,Howanimalstudiesplaytheirroleinabiomedicalresearchrevolution.

Cancer

In the last decade, there has been a significant improvement in the survival rates of many types of cancer, and studies in mice have been among the **most valuable tools used**.

A major advance is the first approved treatment for breast cancer – the **monoclonal antibody, Herceptin** – which was only developed due to initial research in mice and other animals such as rats and hamsters. These studies laid the foundations for the discovery and understanding of the HER2 protein, which can be targeted to reduce tumour growth. Translated into the clinic, studies of breast cancer patientshasshownthataddingHerceptintochemotherapy improvessurvivaland keepscancerat bayfor longer compared to chemotherapy alone.

Some of the cancer breakthroughs that have resulted from research in mice include the development of a type of**immunotherapy cancer treatment**, which usesthe body's immune system to attack and kill cancer cells (called immune checkpoint inhibitors). The researchers who pioneered this treatment, JamesAllisonand Tasuku Honjo, won the 2018 Nobel Prize in Physiology or Medicine.

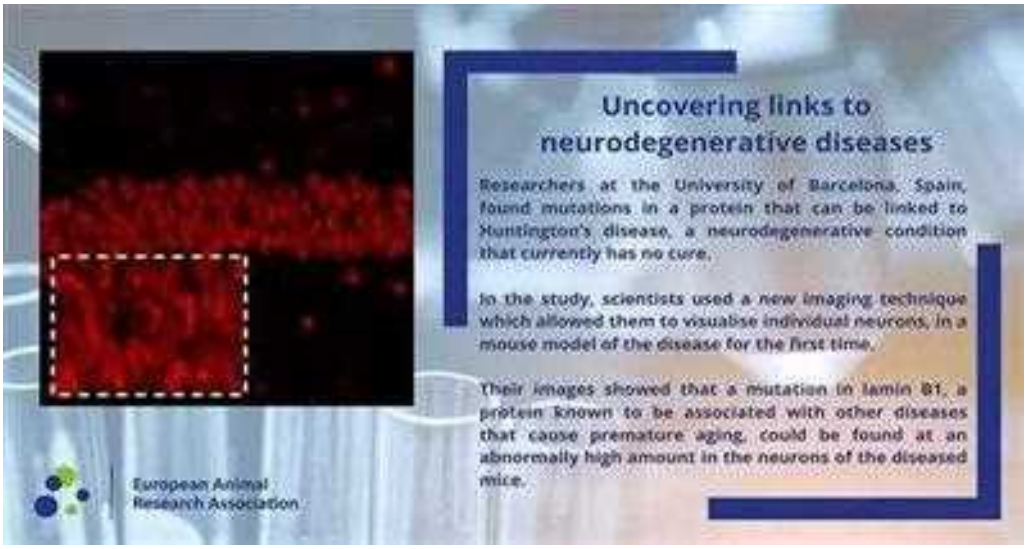
Meanwhile, ongoing cancer research is using mice to help in a broad range of areas, for instance research at EPFL in Lausanne, Switzerland, has revealed a new way to stop **lung cancer tumours** from becoming resistant to treatment. And the University of Bristol, UK, used mice to show that a cancer drug can be repurposed to help withheart attack recovery.

Alzheimer'sdiseaseanddementia

Micecanprovideimportantinsightsintothe**mechanismsofAlzheimer's**,the mostcommontypeofdementia,forexamplebyshowinghallmarksofthe disease,suchasthelossofneuronsinthebrain,orsymptomslikememoryloss, asinvestigated by institutions including theInstitute of Biomedicine of Seville, Spain. Our understanding of other neurodegenerative conditions, such as **Huntingdon's disease**(seeboxbelow),havealsobenefitedfrom the useofmice.

Mice not only allow researchers to explore new treatment options, but also to assess the **potential success of drugs and compounds** before they are trialled in humans – so that they don't cause harm or work differently to how we expect them to.

However, mice are not always the best models for the human brain, especially when it comes to biology.Monkeysare usually used instead as their brainsmore closelyresemble oursand share many of the same features.

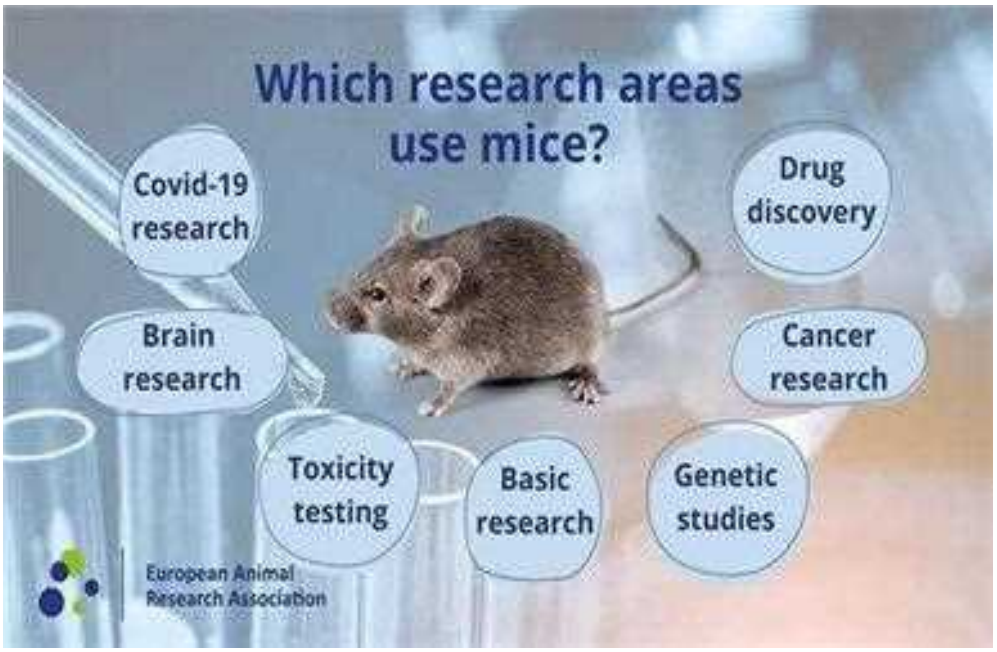


Otherdiseasesandconditions

Research into a range of major conditions, such as**asthma**,**depression**,**lungconditions**and **obesity**, have involved the use of mice. Mice have also been used to understand**healthphenomena**,includingoneofthebiggestglobalthreats,**antibiotic resistance** – for example in a 2022 study by EARA member the Luxembourg Center for Systems Biomedicine – and the effects of zero gravity in space.

Basicresearch

One of the main areas where mice are used is in studies done to gain a**fundamental understanding of the body**. This includesthefunctionsofdifferentgenesand proteins, the differences between the states of health and disease, anatomy and physiology,andhowbiologicalinvaderssuchasvirusescauseinfectionandimpact our health. Researchers can then take this informationandbeginto**applyitto humans**toprovideinsightsintohowwegrow,ageorbecomeill.Astudy includingtheMax Planck Institute of Molecular Cell Biology and Genetics,Germany, showed howgenesworktoinfluencethe**developmentofdiabetes**inmice,for example.



Aremicebeingreplacedorusedinsmallernumbers?

UnderEU Directive 2010/63, researchers mustuse**non-animalalternative methods** whenever they are possible, with the ultimate goal of replacing animals in research entirely. Research animals in the EU can also only be used if there are**no suitable alternatives**. The 3Rs principle (Replace, Reduce, Refine) is an ethical framework for minimising, or avoiding the use of animals in research and is a standard practice in research around the world.

Newapproachmethodologies(NAMs),suchasstudyingcellsinthelab(invitro),lab- grown mini organs (organoids) and natural or engineered tissues grown inside computer chips (organs-on-chips), which mimic human systems and physiology, are emerging as alternatives to animal studies. When these non-animal methods are not possible, the use of mice can also be reduced by replacing them with other animals, such as zebrafish or fruit flies.

There are moves towards**reducing the numbers of GA mice**, for example by freezingthe eggs, sperm or embryos from GA mouse lines, so that they can be accessed at a later date. There is also increased sharing of these strains of mice between researchers, meaning that fewer are ultimately used in the long term.

Credit:UniversityMedicineatJohannesGutenbergUniversityofMainz Is

research on mice and rats the same thing?
Rats are also a very common animal used in biomedical research alongsidemice,but even thoughthetwomightseemsimilar,therearesome importantdifferenceswhen it comes to what they bring to research. Mice and rats have different cognitive and social behaviours, and **react differently to stress, handling, and certain drugs and substances**, all of which mean that one is more suitable over the other, depending on the type of research. Rats are more intelligent than mice, for example, making them better modelsfor conditions like addiction.

TheLaboratoryRatasanAnimalModel forOsteoporosis Research

Osteoporosis is an important systemic disorder, affecting mainly Caucasian women, withadiverseandmultifactorialetiology.Alargevarietyofanimalspecies,including rodents, rabbits, dogs, and primates, have been used as animal models inosteoporosis research. Among these, the laboratory rat is the preferred animal for most researchers. Its skeleton has been studied extensively, and although there are several limitations to its similarity to the human condition, these can be overcome through detailed knowledge of its specific traits or with certain techniques. The rat has been used in many experimental protocols leading to bone loss, including hormonal interventions (ovariectomy, orchidectomy, hypophysectomy, parathyroidectomy), immobilization, and dietary manipulations.

The aim of the current review is not only to present the ovariectomized rat and its advantages as an appropriate model for the research of osteoporosis, but also to provideinformationaboutthemostrelevantageandbonesiteselectionaccording tothegoalsofeachexperimentalprotocol.Inaddition,severalmethods ofbonemass evaluationareassessed,suchasbiochemicalmarkers,densitometry,

histomorphometry, and bone mechanical testing, that are used for monitoring and evaluation of this animal model**in preventive or therapeutic strategies for osteoporosis**.

Osteoporosis is a multifactorial skeletal disease, characterized by reduction in bone mass and disruption of the micro architectural structure of bone tissue, resulting in loss of mechanical strength and increased risk of fracture.

The disorder can be localized or involve the entire skeleton. Generalized osteoporosis can be primary (postmenopausal and senile) or secondary. In the EuropeanUnion,osteoporosis is aleading cause of mortalityand morbidity in the elderly and a key factor in the high cost of medical care.

Although osteoporosis usually makes its appearance late in life, and age is amajor risk factor, its roots can be tracked back into adolescence. Particularly during periods of rapid bone growth, dietary calcium levels are of high importance.

Other factors that contribute to the pathogenesis of osteoporosis are lifestyle and geneticandhormonalattributes.Reducedphysicalactivityincreasestherateof bone loss, and muscle contraction is the prevailing source of skeletal loading. Regarding hormonal factors,women, especially in the decade after menopause, can show a severe reduction of bone mass, thus explaining the high incidence of osteoporotic fractures in women compared with men.

The multiple factors implicated in osteoporosis, its obscure pathogenesis, the dramatic decline in quality of life, high incidence of the disorder (especially in postmenopausal women),financial cost, and high mortality, make the need for further experimentation in animal models imperative.

Experimentalresearchcanimproveourunderstandingofpathogenesisandof the activity of pharmaceutical agents in the prevention or treatment ofthe disease. Although many aspects of the disorder have been revealed,othersremain unclear,including the mechanisms involved in calciumhomeostasis in the extracellularspaceanditseffectonbonephysiologyanddiseaseandthecell and molecular pathways triggered after mechanical loading to orchestrate bone renewal.

Current research is focused on new therapeutic possibilities targeting the osteolytic enzymes of the osteoclast and the mechanisms activating bone progenitor cells and those controlling apoptosis as new potential treatments.

Many therapeutic advances in the management ofosteoporosiswerestudied first in diverse animal models and then entered clinical practice.

All of these models should fulfill similar basic criteria: they must comply with national and local ethical and legislative considerations, be accessible to experimental centers, be easy and safe to handle, have a low cost of acquisition, require little maintenance, reliably reproduce the disease and the biological material to be examined should be readily available.

Laboratory rats meet most of these criteria. In addition, the availability ofdetailed knowledge of the rat skeleton and protocols for rapid induction of osteopenia, have increased this model's popularity.

Here we review the advantages and limitations of the use of the laboratory rat in osteoporosis research.

COMMONLABORATORYANIMALS–INICMRNAREBR

Nowadays, widely used hygienic monitoring programs and sanitation procedureslead to enormous improvement of the microbiological quality of laboratory animals, producing breeding colonies, which are free of pathogens and even free of most opportunistic pathogens.

Undoubtedly, modern**Rodent research management**has been improved and enabled the breeding of Immuno-compromised animal models without microbial induced diseases.

Physiologically and anatomically there are similarities between the humans and animals at organs and organ systems, which function in the similar fashion.

This similarity makes animal idealfor the study and developmentof products and techniques of humans.

By using laboratory animals various discoveries have been made such as diphtheria and polio vaccine, insulin for the treatment of diabeties mellitus, heart valve replacement, antibiotic therapy, manic depressive drugs, etc

CLASSIFICATION OF LABORATORY EXPERIMENTAL ANIMALS IN RODENTBREEDING FACILITY

RODENTS

NON-RODENTS

MISCILLANEOU

S**RODENTS:**

Rodentsaremammals

Characterizedby**upperandlowerpairsofever-growingrootlessincisorteeth.**

Largestgroupofmammals–approx(4660-species)

Mostcommonlyused:**Mouse.Rats**,Guineapig,Gebril,Hamster,etc.

RODENTBREEDINGFACILITY

Dedicated for breeding of SPF quality rodents and lagomorphs such as rats, mice, rabbits, hamsters and Guinea pigs.

ThisisClass1,00,000/ISO8facilitywithcontrolledenvironmentalconditions (temperature : 22 ± 20C and 40-70% humidity).

InICMRRats,miceandrabbitsareusedforResearch.

Facilityhasdedicatedlabsfor**healthmonitoring,geneticmonitoring, preclinicaland vaccine testing services.**

LaboratoryRodents

Mouse:BALB/C,C57BL/6(Black),SWISSALBINO

Rat: WisterandSpragueDawley

NON–RODENTS

GUINEAPIG: Dawkins heartly

Rabbit:NewzlandWhite(NZW)

Breeding Methods

1. Inbreeding–closerelation
2. Outbreeding
3. Randombreeding
4. Selectivebreeding

SWISSisthepartofBALB/Canditisoutbreedorrandombreed.

BALB/C and C57BL/6 are inbreed.**Chromosome number**:

1. Rats:48
2. Mice:20
3. Rabbits:44
4. Guineapig:64

Gestationperiod:

Mice(C57bl6):18.5days

Gestationlabrats:21-23days

Littersize:

C57BL/6(morethan10)

BALB/C(8-14)

SWISS(15 to16)**CagingSystems** :

1. BarrierSystem
2. ConventionSystem
3. Individual Ventilation Caging System

Weaning Period:

21daysinmouse

15daysinguineapig

3monthsin rabbits

MatingMethods:

1. Monogamous
2. Polygamous
3. MassMating
4. HandMethodMating
5. HaremMating

BeddingMaterialsForAnimals:

PaddyHusk,CornCobb,WoddenChips,CelluloseMembrane,RawPaper

TYPESOFEXPERIMENTALANIMALSUSEDINLABORATORY

Animalsmostlyusedin**RODENTBREEDING/EXPERIMENTATION**facility- ICMR NARFBR

1. MOUSE
2. RAT

MOUSE:(MUSMUSCULUS)

Themost**commonmammal**amonglaboratoryanimals-**MICE**

CHARACTERSTICS

1. Micearesmalllaboratoryanimalsweighingabout(18-30g).
2. Theyareverysensitiveandconsumesmalldosesofdrugs.

3. Theycanbeeasilyhandled.
4. Drugs are injected through intraperitoneally or intravenously into one of their superficial tail veins.

AREASOFRESEARCH

The following are the research areas where the above mentioned animals are usedas experimental animals.

1. Incancerandgeneticresearch.
2. Inacutetoxicity studies
3. Inscreeningofvarious drugactivities speciallycentralnervous system(CNS) activity.

MICESEXING:(DISCRIMINATESEX-MALEANDFEMALE

The distance between the anal and genital orifices is greater in –male compared to –female.

HANDLINGANDRESRAINTOFMICE

Gentlybut firmly

Weardisposalgloves

Washyourhandspriortoandafterhandling

Wearacleanlaboratorycoat.

GENTICALLYMODIFIEDMICE

Knockoutmouse

Knock in mouseKnock out mouse

It is genetically engineered mouse in which researchers have INACTIVATED or KNOCKED OUT , an existing gene. This is the most important animal model to **studythe role of genes that have been sequenced but whose functions have not been determined.**

This causeschangesin mousephenotype(appearance,behaviour,physicaland biochemicalcharecteristics)

Causing a specific gene inactivation, and observing any differences from normal behaviour and physiology, we can infer its probable function.

May also be a useful experimental pathological model to test the efficacy of new drug.

RAT:(RATTUSNOVERGICUS)

Laboratory rats play an important role in biomedical research across range oftherauptic areas.

These arecloselyrelatedtothehumangenome.

CHARACTERISTICS

1. Ratshavealargerweightthanmice(about300-500g)
2. Theycanwithstandlongperiodsofexperimentationunder anaesthesia.
3. Theycanbeeasilyhandlediftreatedkindly.
4. Drugs can be injected through Intravenously(I.V) into their tail veins. Subcutaneously(S.C) , intramuscularly(I.M)
5. Theycanalsobegiven drugsorallybymeans ofstomach.

PROPERMETHODOFHANDLING:

Toinitiallyrestrainarat,thehandlershouldbegentlygraspitaroundthe shoulders.

Thehandlersthumbcanthenbeplacedunder therats mandibletopreventbites, and the rats hind limb scan can be supported with the other hand.

Restraintshouldbefirmbutnottootightasthiswillimpedetheanimal's respiration.

Gastricgavageorallyinrats.

Usedinresearch:

Mostlyusedrat-(ALBINOWISTARRAT)

1. Tostudybehavorialandpsychologicalstudies.
2. Cardiovascularstudies.
3. Neuralregeneration.
4. Spacemotionsickness.

5. Usedintoxicologyandpharmacologystudies.

STANDARDOPERATINGPROTOCOLFORLABORATORY RODENTS USED INRESEARCH

Introduction

The intent of this Standard Operating Protocol (SOP) is to describe acceptable methods of transport, housing, care, enrichment, anesthesia, and euthanasia ofrats and mice.

This SOP is intended for personnel that use rodents in research , who care for animals in the Animal Care Facility.

This procedure is approved by the UWSP Institutional Animal Care and Use Committee (IACUC; October 31, 2011)

RequirementsofpersonnelimplementingthisSOP

The principal investigator or lead instructor must ensure that all individuals responsible for rodent care and/or use are familiar with all elements of this SOP as well as additional requirements outlined in individual protocol.

Safety

Personnelshouldbeawareofsafetyequipmentlocationsandrecommendationsfor use. General safety protocols can be found on the UW-SP Environmental Health and Safety website: http://www4.uwsp.edu/ehs/

No food or drink intended for human consumption is allowed in thefacility,except in the administration office.

If any maintenance issues arise such as extreme changesintemperatureorroom flooding, maintenance can be reached. In case of emergency, personnel should call Campus Security .

The Animal Care Facility maintains restricted locked access. The surgery room and all rooms containing rodents must be locked before personnel leave the facility. Only authorized personnel are admitted into the facility. Visitors must be supervised at all times.

Transportation

Personneltransportingrodents,whetherintra-orinter-institutionally,must ensure the safety and comfort of the animal(s) at all times.

Transportationmediamustprovideadequatefood,water,temperature, humidity, comfort, and overcrowding must not occur

Deliveryofanimalsmustbecoordinatedtooccurduringbusinesshours.

RodentInventory

Rats and mice are to be housed in separate rooms. Identification cards must include source of animals, date of arrival, date of birth, species, sex, name or other identification.

If anyRatsshouldbehousedtwopercageunlessprotocolspecifiesotherwise.

If a cage with two large rats is becoming soiled in less than a week, or contains animals who are causing each other harm, then those animals should be separated.

Mice, baby/juvenile rats may be housed more than two per cage. Births and deaths should be recorded on daily log sheets.

Husbandry

All personnel should have working knowledge of cleaning, feeding, watering,andenrichment schedules

Onlytrainedworkersareauthorizedtoimplementthesetasks.

Logsheetsmustbecompleteddailyforeachroomthathousesrodents.

Personnelmustensurethatappropriatelysizedcagesforeachanimalareused to prevent escape.

1.7.a.Observation&monitoring

Eachagemustbecheckeddailyforwaterandfoodlevels,animalhealth,andanimal births or deaths.

Logsheetsmustbecompleteddaily.

Theacceptable temperature range for rats andmice is between 68and79degrees Fahrenheit (20-26 degree Celsius)

Relativehumidityshouldbebetween30%and70%.

1.7.b .Feeding

Allrodentsshouldhaveadequatefoodintheirfoodhopper(atleast½cup).

Foodlevelsmustbecheckeddaily,andmorefoodshouldbeaddediflevelsare observed to be low.

Aclosedfoodcontainershouldbeavailableineachrodentroom.

If food containers are empty, personnel may obtain more food from the supply room. The date that the container was refilled should be logged on the food container.

Recommendation from the feed manufacturer should be followed regarding shelf life and storage.

Additional food may be provided to rodents as 'treats' at the caretaker's discretion.

If this food is stored in the Animal Care Facility, it must be labelled "not for human consumption" and dated.

1.7.cMortality&removal

Mortalities should be removed daily from each cage, recorded with the daily log and on the Euthanisia/ Natural Death log sheet, placed in sealable bags, labelledwith the date that animal was observed to be dead, and placed in the freezer.

Rodentsthataresafeforavianorreptileconsumptionmaybedonatedforthat Purpose.

All other animals are placed in a cremation bag and delivered to the Biology stockroom.

1.7.d Cagecleaning

Cages are cleaned at least once per week. Cleaning consists of replacing cage,bedding, water bottle, sipper, and any toys or housing inside cage.

The wirecagetopsmustbesanitizedatleastoncepermonth.

Dirtyhousingequipment,waterbottles,sippers,andtoysaresanitizedinthecage washer.

Beforecagesareplacedinthecagewashertheymustberinsedandscrubbed out, using soap if very dirty.

Ifnewspaperisusedinplaceofbeddinginanextra-largecage,itmustbe replaced before rotating animals in that cage, and the pan must be disinfected.

After bedding is disposed of in waste container, waste should be emptied in the loading dock dumpster.

1.7.eRoomand facility cleaning

Floors in the Animal Care Facility should remain generally free of bedding and other debris.

Foodspillsmustbecleanedupimmediately.Regularfacilitycleaningis scheduled as follows:

1. Floors should be swept and mopped weekly at a minimum, and more often as needed.
2. Cageracksshouldbesanitizedoncepermonth,andsweptasneeded.
3. All areas should be kept free of clutter. Vent filters in rooms containing rodents should be changed every two months, and those in all other rooms should be changed biannually.

1.7.fDiseasecontrol

Personnel handling rodents or any associated equipment should wash their hands thoroughly upon completion of work or more often as needed.

All housing, feeding,and watering equipment must be disinfected before used for adifferent animal.Foodandwaterthat has beenusedforone animal(orset of animals) must not be given to another animal.

Personnel should ensure that no open wounds are present on their hands before handling the animals.

Disposableglovesareavailableforuseinthecagewashroom.

.g.Quarantine

Rodents suspected of having a contagious disease should be separated from other animals. Quarantine areas should be managed according to rigorousinfectiousagent control practices.

Enrichment

Enrichment is highly encouraged and should be provided as often as possible. Some protocol may specify that certain animals are to receive no enrichment.

Thefollowingarerecommendationsforenrichment:

Twomiceper cage

Housing structures inside cages, which could include cardboard boxes, PVC elbows, jars, etc.

Wheels

Freshherbsforscentstimulus

Rotation of rats to large cage (rats from more than one cage should not be mixed in the large cage)

Treats: peanut butter, bird seed mixed in (clean) bedding, fruit, vegetables, bread, cereal, etc. Only asmall amount of perishable food such as fruit should be placein a cage at one time.

Plasticbottleswithfoodinsideor‘congs’containingfood(theyhavetoworkto get it out)

Miceshouldbeprovidedwithnesting squares

Ratsshouldbehandledonaregularbasis

Anesthesia

Anesthesia should be used before performing procedures that may cause more than momentary mild discomfort.

Carbondioxideandisoflurineareapprovedforanesthesia.

Personnelshouldadheretoproceduresoutlinedinapplicableprotocol

Euthanasia

Euthanasiaofratsandmiceareasfollows:

Carbon dioxide halothane, isoflurane, sevoflurane and Desflurane, with or without nitrous oxide

Regardless of method used, euthanasia must be performed in the provided container under the fume hood in the surgery room.

Personnel performing euthanasia shall perform checks to determine whether the animal is expired while the animal remains in the chamber.

PersonnelperformingeuthanasiashallfilloutheEuthanasialog.

Any personnel performing euthanasia must receive appropriate training to perform the

Procedure,adheretoIACUC-approvedprotocolsandinstitutionalpolicies.

1.11.Disposalofcarcasses

Upon death, all animals should be placed in the freezer. Those that are safe for animal consumption may be donated and those that are not must be delivered to the stock room in a cremation bag for incineration.

INTRAVENOUSADMINISTRATIONINRODENTS:

Themostaccessiblevesselsforintravenousadministrationinrodentsareveinsthatrunthel lateral aspects of the tail.

ContacttheIACUCofficeforone-on-onetrainingsessions.

Supplies:

Sterile27-30gaugeneedlesfor mice

Sterile25-27gaugeneedlesforrats

500uLto 1mlsyringeformiceis recommend

1mlor3mlsyringesforrats

Heatingdevice

Gauzesponges

Isoflurane,AnesthesiaSystem(recommended)

Note:Itishighlyrecommendedthatnewusersofthistechniqueanesthetizetheanimalswhilec procedure until proficiency is obtained.

ProcedureSteps:

Weigheachanimalbeforeinjection

Upto1%oftheanimalsbodyweightinthevolumecanbeadministeredperinjection.

1. Recordbodyweightsandagentvolumestobe administeredfor each animal.

2. Priortoinjection,warmanimalfor5-10minutestodilatetheveins.Animalmaybe warmed by placing the animal in a commercially available warming box, brass restrain or by using a warm water circulating pad placed under the cage. These are the safest and most effective ways to warm rodents. Ifan overhead heatlamp is used, extra care must be taken to prevent overheating the animal.

3. Itisrecommendedtolightly anesthetizethe animal.

4. Whenanesthesiaisnecessary,positiontheanimalonitssideonarodentsafe heat source or circulating warm water pad to maintain thermoregulation during anesthesia.

5. Conscious animals need to be restrained using a commercially available restraint deviceofappropriatesize(seebelow).Aprewarmedbrassrestraintprovidesheat forvasodilation,reducingtheneedforprewarmingmiceinacage.Thedurationof

the restraint should be kept to a minimum, and the equipment washed frequently to prevent pheromone-induced stress or cross contamination. Rodents sometimes spinin the restrainer; be sure to confirm to orientation and location of the lateral tail vein before performing injections.

6. Hold the syringe with the dominant hand near the bottom so that the remaining fingers are near the plunger and can easily push the agent into the vessel without disrupting the needle in the vein.

7. Syringes should be prepped with no air bubbles. One syringe and needle are recommended per mouse/rat. Needles should be sharp and replaced after two attempts.Insertthe needle(smallgauge, 27-30for mice and25-27for rats),bevelup, intotheveintowardsthedirectionofthehead.Keeptheneedleandsyringeparallel tothetail.Aspirationisnotadvisedasitmaycausetheveintocollapse,butaflash ofbloodinthehubofftheneedlemaybeseenwhenfirstplaced.Properplacement may not be verifiable until injection occurs, but when placed correctly the needle should advance smoothly into the vein.

8. Slowly inject. If there is resistance and/or a blister or white area appears above the needle on the tail, the needle should be removed and re-inserted above the first site.

9. Removetheneedleandapplygentlecompressionuntilbleedinghasstopped.

10. Iftheanimalwasanesthetized,monitortheanimalduringtherecoveryprocess.

11. Return animals to their cage and observe to make sure that bleeding has notresumed.

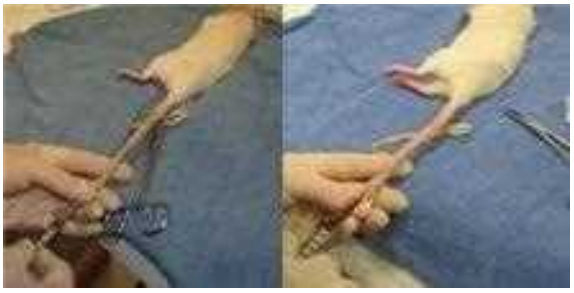
Note: With brown or black mice and rats, an additional light source may be necessary to aid in visualizing the tail veins. Rats have scalesmaking thevein difficulttosee,especiallyinolderadults.Thescalesareremovedbygentlycleaning thetailwithasalineorchlorhexidinesolutionmakingtheveinsmoreapparent-wipe in the direction of in the direction of the scales to avoid irritation to the tail.

Mouse:

Grasp the tail at mid-length or at the distal (further down the tail) end. The index and middle fingers of the non-dominant hand are placed around the tail above where the needle will be inserted (digital pressure will act as a tourniquet). The lower part ofthe tail is held between the thumb and ring finger below the injection site. Put slight tension on the tail by applying pressure with both setsoffingers. Needle should enter the vein at a shallow depth, keeping syringe and needle parallel to tail. Release pressure to the proximal fingers before administering the agent into the vein. No resistance should be felt when depressing the plunger.

Note: With mice, elevating the animal about 4-6 inches off the table may be helpful with keeping the needle and syringe parallel to the vein.

Rats: A tourniquet is used to constrict the vein to allow visualization and access to the vein for injection mid-length or at the distal (further down the tail) end. A tourniquet is made with a rubber band wrapped around the top of the tail and held together firmly with a hemostat. The tourniquet is released before the agent is administered into the vein.



Agents:

This procedure recommends anesthesia. All agents administered to animals should be listed in the "Agents" section of the RIO IACUC protocol.

AdverseEffects:

Adverseeffectsshouldbelistedinthe“AdverseEffects”sectionoftheRIO IACUC protocol. Examples of potential adverse effects include: Peri-vascular irritation, Blood loss .

LABANIMALHEALTHMONITORING

Monitoring the health of laboratory animals is one of themany responsibilities researchers bear when performing their critical research.

Our portfolio of lab animal health monitoring services, including pathology, serology, PCR infectious agent testing, and more, can help you detect and eliminate any pathogens that could negatively influence your studies.

Ourprotocolsinlabhealthmonitoringinclude

1. pathology
2. serology
3. microbiology
4. parasitology
5. pcrinfectiousagenttesting
6. immunoassays-(ELISA)

To effectively detect pathogens, it's best to use multiple lab animal health monitoring testing modalities at various levels within a research facility.

We are always looking to identify and incorporate the mostanalytical sensitive and specific technologies in order to provide you with the most accurate reproducible results.

MATERIALSANDPROCEDURESOFLABORATORYANIMALS:

1. **Streakplatemethod**
2. **Gramstaining**
3. **Preparationofvariousmedia**
4. **Polymerasechainreaction**
5. **Elisa**

STREAKPLATEMETHOD

INTRODUCTION:Streaking is a technique used in microbiology for the isolation ofsingle colonies of microorganisms, either from a mixed species or from the same species. This technique is mostly applicable to bacteria but is also used for some yeasts. It is an old technique that hasbeen in use since the time of Rober Koch. It wasfirst demonstrated byLofflerand Gaffky in Koch's laboratory.

PRINCIPLE : The streak plate method is based on the principle of dilution. It can be described asarapidqualitativeisolationtechnique.Themaincriterionofisolationisto obtainareducednumberofcolonies.Inthistechnique,aloopfulofculturespreadon an agar platetogetindividualcellsfarapartenoughfromeachother.The streaking method gradually dilutes the inoculum such that the bacterial cells can be

countedascolonyformingunits(CFUs).

MATERIALSREQURIED:

Petridish—sterile,disposable,90mmdiameter.

Media—e.g.f2.

Agar—Bacto-Agar,Difco.

Wireloops—nichcromeorplatinum.

Bunsenburnerorsmallflame.

Parafilm.

TypesofStreakPlateMethod:

Quadrant Streaking: This is the most common method of streaking, where the petri dish is divided into four quadrants and then inoculated. It is also known as a four-quadrant streak. A loopful of inoculum is taken and streaked such that the first quadrant contains the highest concentration of the inoculum, followed by the second quadrant, third quadrant, and fourth quadrant. This is a discontinuous method where the loop is sterilised after streaking in every quadrant. By the time the fourthquadrant is streaked, the inoculum is diluted enough to give rise to individual colonies.

T-Streaking: In this method, the petri dish isdivided into three quadrants by drawingtheletter‘T’. Similarto four-quadrantstreaking,itisa discontinuousmethod where eachquadrantisstreakedafterloopsterilisation,andthequadrantthatisstreakedlast gives isolated colonies.

Continuous Streaking: In this type of streaking, the inoculum is spread from one edge to the centre of the plate.The plate is rotated 180°, and the remaining portion is streaked without sterilizing the loop.Another form of continuous streaking is used for diagnostic purposes.The petridish is divided into sections,and differentcultures, suchasurine,sputum,pus,etc.,arestreakedineachsectiontogetmaximum output.

RadiantStreaking:Inthisstreakingmethod,theinoculumisspreadononeedgeof

the petri dish. From the edge, vertical lines are streaked in the upward direction. Next, the vertical lines are streaked horizontally to obtain pure isolated cultures.

StreakPlateMethodProcedure:

-Steriliseallltheinstruments,flasksandmediathatarerequiredforthestreaking procedure.

-Cleanyourworkareausingadisinfectanttominimiseanycontamination.

-Setupthebunsenburnerinyourworkareacarefully.

-Washyourhandswithanantisepticsolutionbeforehandlinganymicrobialsolution.

-Label the petri dish with all important information, such as your name, date, media used and the culture being inoculated.

-Topickupthesample,youcanuseeitherametalloopordisposableplasticloops.

-Aloopfulofsampleisstreakedonthefirstquadrantinaback-and-forthmotionon the

-Sterilisetheloopbyheatingitinthebunsenburnerifusingametalloop.

-Streaktheotherthreequadrantsbyasimilarmethod.

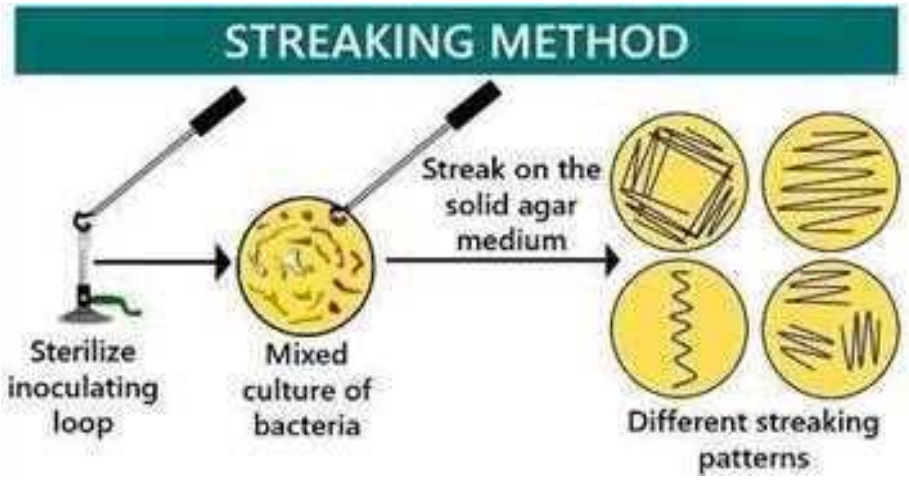
-Closethelidoftheplateafterstreaking,andstorethedishupsidedowninan incubator with optimal temperature.

AdvantagesoftheStreakPlateMethod:

It is one of the most popular techniques used to obtain isolated colonies of bacteria.

It finds a great application in biotechnology as it can be used to identify transformed bacteria from non-transformed bacteria by adding an antibiotic to the growth medium.

Italsofindsgreatapplicationindiagnosticpurposes.



STREAKPLATEMETHODUSEDINICMR

- To check the presence of different types of micro organismsinsideand outside the body of animals which are grouped under Rodents, Small Ruminants ,Canines ,Primates and Equines.

GRAM'SSTAINING

INTRODUCTION: Staining, in microbiology, can be defined as a technique which is used to enhance and contrast a biological specimen at themicroscopic level. Stains and dyes are used to highlight the specimen at the microscopic level to study it at higher magnification for histopathological studies and diagnostic purposes.

However, staining is not just limited to biological specimens, it can also be used to study the structure of crystalline polymers.

PreparationoftheBiologicalSpecimen:

The preparation of the biological specimen to be analysed underamicroscope depends on the type of staining. Given belowaresome procedures that are carried out.

WetMounting:Livingbiologicalspecimensaremountedonaglassslidewith

water andspecificstains.

Fixation:Itis amulti-stepprocess whichisdonetopreserve theshapeof cells and tissues. Heat fixation is done to kill and adhere the specimens. Chemical fixation is done to generate strong bonds and increase the rigidity of the samples. Common chemical fixatives used are formaldehyde, picric acid, methanol and ethanol.

Mordant: Mordants are chemical agents that are used along with dye to make the specimen stainable, which otherwise is unstainable. Mordants are of two types:

Basic Mordants: They react with acidic

dyes.**AcidicMordants**:Theyreactwithbasic

dyes.

When staining is done with the help of mordants, it is known as indirect staining. On the other hand, when staining is done without the help of mordants, it is known as direct staining.

Permeabilisation: This procedure involves treating thespecimenwithasurfactant that dissolves the cellmembraneallowingeasystainingwiththe dye.

TypesofStainingTechniques

Gram'sstaining:This stainingprocedureisusedtoidentifybacteriabasedon their cell wall composition. There are two types of Gram's staining, and the bacteria can be divided into gram positive or gram negative bacteria. It uses crystal violet for the staining of cell walls, iodine as the mordant and safranin or fuchsin as the counterstain

BiologicalStains

Someofthemostcommonlyusedbiologicalstainsarelistedbelow:

-Acridine orange :It is a fluorescent cationic dye that is selective to nucleic acids. It is used during the cell cycle to analyse DNA molecules.

-Coomassieblue:Itisusedingelelectrophoresistostaintheproteinsblue.

-Crystalviolet:ItisusedinGram'sstainingalongwithiodinetostainthe bacterial cell wall in purple colour.

-Eosin:Itisusedasacounterstaintohematoxylinthatimpartsaredcolourto the cytoplasm and its components.

-Ethidium bromide: It provides a red-orange fluorescent stain to the DNA after intercalating with the molecule.

-**Iodine**:ItisusedasamordantinGram'sstaining.

-**Malachitegreen**:Itgivesablue-greencolourwhenusedas acounterstain against safranin that is used in endospore staining.

-**Methyleneblue**:Itisusedtostainanimalcellsbecauseitenhancesthenuclei.

-**Safranin**: It is a red cationic dye that is used as a counterstain in both Gram's staining and endospore staining.

AREASOFRESEARCHINICMR:

Different samples of animals such as feces , hair, and skin scrap are send to the microbiology lab fortestingthepresenceofmicrobes.Aftertheplatingtechnique the grown microbes are examined by using this staining technique . We found **SALMONELLA TYPHI** which is a gram negative bacteria observed in pinkcolour in the above slide.

PREPARATIONOFVARIOUSMEDIA

PreparationofMacConkeyAgar:

Purpose: Mac Conkey agar is a differential medium to distinguish lactose fermenting gram negative bacteria fromnon lactosefermentors; lactosefermentorsarecoloured pink, while non lactose fermentors are pale. It is appropriate for stool, urine, urogenital specimens, throat swabs, ear and eye swabs, woundswabs,sputum, aspirates, cerebrospinal fluid.

Procedureforpreparation:

-Suspend51.1gofpowderin1litreofdistilledordeionizedwater.

-Heatuntilcompletelydissolved.

-Sterilizeinautoclavedat121Cfor15minutes.

-Coolto45-50C.

-Pour15-20mlofthereadymediaontopetridishes.

-Leavestandingforthirtyminutestosolidify.

-PerformsterilitytestingasdescribedinSection7.

-Labelthebottomofeachplatewithdateofpreparationandbatchnumber.

-Storetheculturemediaplatesupsidedownat2-80Csealedinplasticbagsto reduce chances

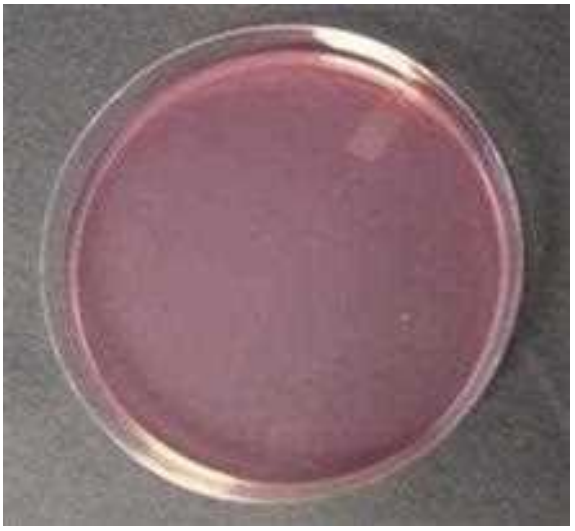
ofcontamination.

-TestSamplesforperformance,usingstable,typicalcontrolcultures.

-**Shelf life**: up to sixteen weeks provided there is no change in the appearance of the medium

tosuggestcontaminationordeteroriation

-pHofthemedium7.2-7.6atroomtemperature



Nutrientagar:

Purpose:Nutrientagarisusedforthecultivationofawidevarietyofnon-fastidious

bacteria. It was originally developed in recognition of the need for a standardized medium for use in the examination of water and waste water, dairyproductsandvariousfoods.Currentlyitisusedasamaintenance

medium for Staphylococcus aureus, Proteus mirabilis, and Escherichia coli. Tube slants are used primarily for the cultivation and maintenance of pure cultures.

Procedureforpreparation:

(i) Suspend23gofthepowderin1litreofpurifiedwater.

(ii) Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

(iii) Autoclaveat121˚Cfor15minutes.

(iv) Coolto45-50C.

(v) Pour15-20mlofthereadymediaintosterile20mlglassuniversaltubes.

(vi) Leave standing for thirty minutes to solidify, resting the tubes leaning at 30˚- 60˚ to

producetheslopeeffectsinthetubes..

(vii) PerformsterilitytestingasdescribedinSection7.

(viii) Labelthesideofeachtubewithdateofpreparationandbatchnumber.

(ix) Storethetubesat2-80Csealedinplasticbagstoreducechancesof contamination.

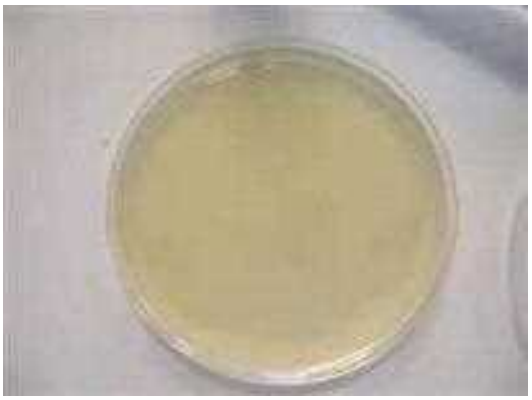
(x) TestSamples of the finishedproduct for performance, using stable, typical control

Cultures .

-Shelf life: up to eighteen months provided there is no change in the appearance of the

mediumtosuggestcontaminationor deteroriation.

-pHofthemedium7.2-7.6atroomtemperature.



AREASOFRESEARCHINICMR:

These mediums are used for the culturing and isolation of pure colonies ofMicrobes which are isolated from the Animal samples.

ISOLATIONOFDNAFROMBACTERIA:

Bacteriaiscollectedfromtheliquidbrothmedium.

Materialsrequired:

-OvernightcultureofcoliinLB

-TEbuffer(10mMtris.Cl,1mMEDTA,pH 8.0)

-10%SDS

-1:1Phenol-Chloroformmixture

-Chloroform

-5NNaCl

-5MAmmoniunacetate

-Icecoldisopropanol

-70%ethanol

ProtocolofDNAextractionfromE.coli:

-Take 1.5 ml of bacterial broth culture (overnight culture of coli in LB) into amicrofuge tube.

-Centrifugeat800rpmfor10minutesat4°Canddiscardthesupernatant.

-Suspendthepelletin400µlTEbuffer.Mixwellbyvortexing.

-Add10µlof10%SDSandmixit.

-Incubatethetubeat37°Cfor1hourinwaterbath.

-Shearthecellsuspension3-5timeswiththehelpof26Gneedle.

-Add500µlof1:1phenol-chloroformmixture.

-Centrifugeat13000rpmfor2minutesat4°

-Transferthesupernatantintoanothermicrofugetube.

-Add500µlofchloroformandcentrifugeat13000rpmfor2minutesat4°

-Transferthesupernatantintoanothermicrofugetube.

-Add25µlof5NNaClandcentrifugeat13000rpmfor10minutesat4°

-Discardthesupernatantandsuspendthepelletin100µlTEbuffer.

-Incubatethetubeat37°Cfor30minutes.

-Add10µlof5Mammoniumacetate.

-Add250µlofcoldisopropanolandincubateatroomtemperaturefor5minutes.

-Centrifugeat13000rpmfor10minutesat4°

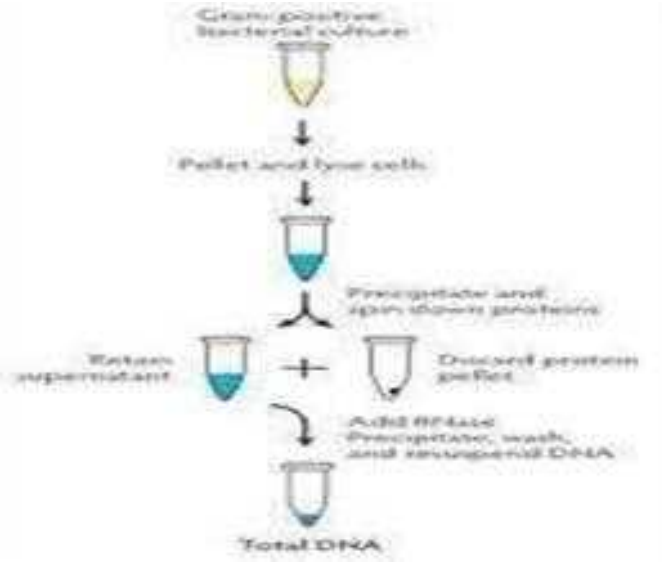
-Discardthesupernatantandwashthepelletwith100µlof70%ethanol.

-Pourofftheethanolandinvertthetubeonacleanabsorbentpapertodrain.

-Allowthepellettoairdryfor5-10minutes.

-Suspendthepelletin100µlTEbuffer.

-Storeat-20°.



PolymeraseChainReaction(PCR)

PCR or Polymerase Chain Reaction is a technique used in molecular biology to create several copies of a certain DNA segment. This technique was developed in 1983 by Kary Mullis, an American biochemist.PCRhas made itpossible to generate millions of copies of a small segment of DNA. This tool is commonly used in the molecular biology and biotechnology labs.

PrincipleofPCR

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA Polymerase synthesises new strands of DNA complementary to the template DNA. The DNA polymerase can add a nucleotide to the pre-existing 3'-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3' prime end of the DNA polymerase.

ComponentsOfPCR

ComponentsOfPCRconstitutesthefollowing:

-DNATemplate–TheDNAofinterestfromthesample.

-DNA Polymerase– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.

-Oligonucleotide Primers- These are the short stretches of single-stranded DNA complementary to the 3' ends of sense and anti-sense strands.

-Deoxyribonucleotidetriphosphate–Theseprovideenergyforpolymerizationandare the building blocks for the synthesis of DNA. These are single units of bases.

-Buffer System– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability.

PCRSteps

ThePCRinvolvesthreemajorcyclicreactions:

Denaturation: Denaturation occurs when the reaction mixture is heated to 94°C for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA.

The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

Annealing: Thereactiontemperatureisloweredto54-60°Cforaround20-40seconds.
Here, the primers bind to their complementary sequences on the template DNA.

-Primersaresingle-strandsequencesofDNAorRNAaround20to30basesinlength. They serve as the starting point for the synthesis of DNA.

-The two separated strands run in the opposite direction and consequently there are two primers- a forward primer and a reverse primer.

Elongation: At this step, the temperature is raised to 72-80°C. The bases are added to the 3' end of the primer by the Taq polymerase enzyme.

-This elongates the DNA in the 5' to 3' direction. The DNA polymerase adds about 1000bp/minute under optimum conditions.

-Taq Polymerase can tolerate very high temperatures. It attaches to the primer and adds DNA bases to the single strand. As a result, a double-stranded DNA molecule is obtained.

-These three steps are repeated 20-40 times in order to obtain a number of sequences of DNA of interest in a very short time period.

AGAROSE GEL ELECTROPHORESIS

Introduction:

-Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode.

-Shorter DNA fragments migrate through the gel more quickly than longer ones. Thus, you can determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known

lengths)

Equipment:

-Castingtray, -Wellcombs,-Voltagesource,-Gelbox,-UVlight source, -Microwave

Reagents:

-TAE(recipehere),-Agarose,-Ethidiumbromide(stockconcentrationof10

mg/mL)**Procedure:**

-PouringaStandard1%AgaroseGel:

-Measure1gofagarose.

-Mixagarosepowderwith100mL1xTAEinamicrowavableflask.SeeTAERecipe.

-Microwavefor1-3minuntiltheagaroseiscompletelydissolved(butdonotoverboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up.).

-Concentration of approximately 0.2-0.5 μg/mL (usually about 2-3 μl of lab stock solution per 100 mL gel). EtBr binds to the DNA and allows you to visualize the DNAunder ultraviolet (UV) light.

-Pourtheagaroseintoageltraywiththewellcombinplace.

-Placenewlypouredgelat4°Cfor10-15minsORletsitatroomtemperaturefor20- 30 mins, until it has completely solidified.

-LoadingSamplesandRunninganAgaroseGel:

-AddloadingbuffertoeachofyourDNAsamples.

-Oncesolidified,placetheagarosegelintothegelbox(electrophoresisunit).

-Fillgelbox with1xTAE(orTBE) untilthe geliscovered.

-Run the gel at 80-150 V until the dye line is approximately 75-80% of the waydown the gel.

-A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.

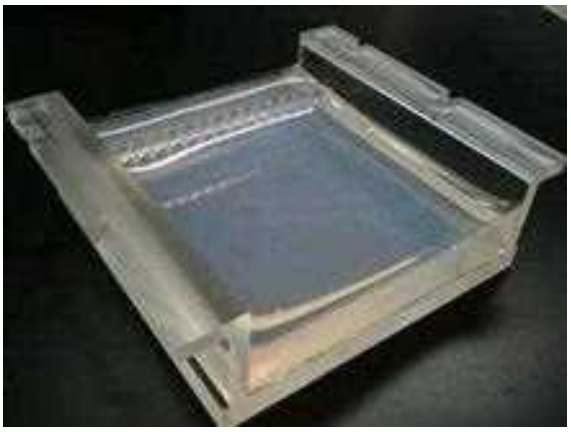
-Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box Using any device that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.

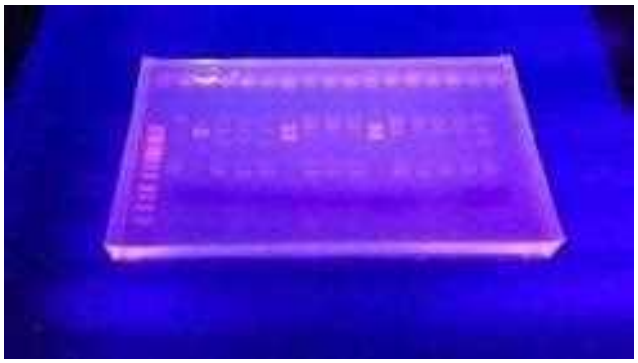
AnalyzingYourGel:

-UsingtheDNAladder inthefirstlaneasaguide(themanufacturer's instructionwill tell you the size of each band), you can infer the size of the DNA in your sample lanes. For more details on doing diagnostic digests and how to interpret them please see the Diagnostic Digest page.

PurifyingDNAfromYourGel:

-Ifyouareconductingcertainprocedures,suchasmolecularcloning,youwillneedto purify the DNA away from the agarose gel. For instructions on how to do this, visit the Gel Purification.





AREASOFRESEARCHUSEDINICMR:

AftertheextractionoftheDNAfromthebacterialcellstheDNAisamplifiedby usingthe PCR technique. After the completion of PCR many cycles are formed , the formed DNA is run under the GEL ELECTROPHORESIS and examined under UV TRANSILLUMINATOR.

ENZYMELINKEDIMMUNOSORBENTASSAY{ELISA}

- ELISAis the basic assay technique,known as enzyme-linked immunosorbentassay (also referred to as EIA: Enzyme Immunoassay) that is carried out to detect and measure antibodies, hormones, peptides and proteins in the blood.

-Antibodies are blood proteins produced in response to a specific antigen. It helps to examine the presence of antibodies in the body, in case ofcertaininfectious diseases.

-ELISA is a distinguished analysis compared to other antibody-assays as it yields quantitative results and separation of non-specific and specific interactions that take place through serial binding to solid surfaces, which is normally a polystyrene multiwellplate.

PrincipleofELISA:

ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide useful measurement of antigen-antibody concentration.

TypesOfELISA:

-ELISA tests can be classified into three types depending upon the different methodsused forbinding between antigen and antibodies, namely:

Indirect ELISA – Antigen is coated to the microtiter

well**Sandwich ELISA** – Antibody is coated on the

microtiter well

CompetitiveELISA–Microtiterwellwhichisantigen-coatedisfilledwiththe antigenantibody mixture.

IndirectELISA:

-IndirectELISAdetectsthepresenceofanantibodyinasample.

-Theantigenisattachedtothewellsofthemicrotitreplate.

-A sample containing the antibodies is added to the antigen-coated wells for binding with the antigen.

-The free primary antibodies are washed away and the antigen-antibody complex is detected by adding a secondary antibody conjugated with an enzyme that can bind with the primary antibody.

-All the free secondary antibodies are washed away. A specific substrate is added which gives a coloured product.

SandwichELISA:

-SandwichELISAhelpstodetectthepresenceofantigeninasample.

-Themicrotitrewelliscoatedbytheantibody.

-The sample containing the antigen is added to the well and washed to remove free antigens.

- Then an enzyme-linked secondary antibody, which binds to another epitope on the antigen is added. The well is washed to remove any free secondary antibodies.

Theenzyme-specificsubstrateisaddedtotheplatetoformacolouredproduct, which can be measured.

CompetitiveELISA:

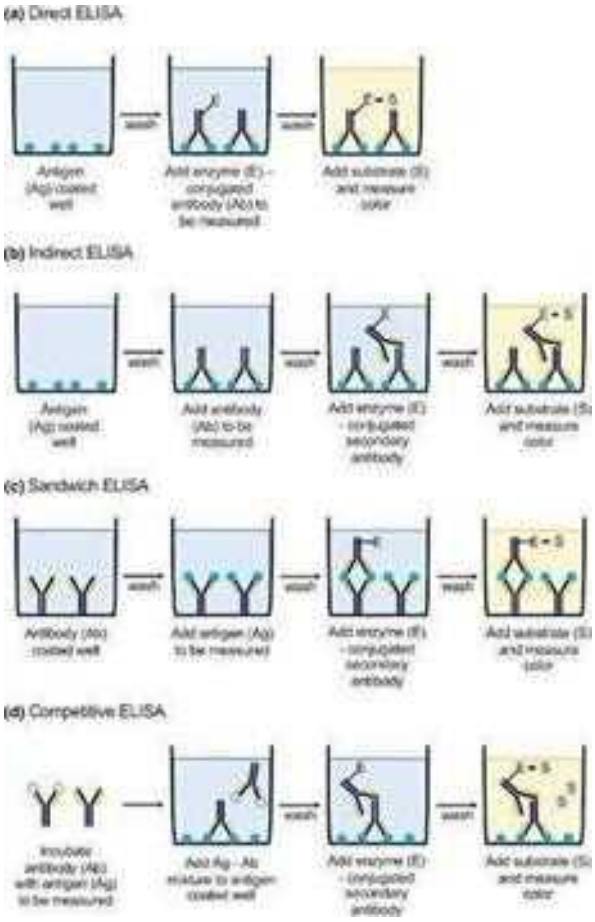
-CompetitiveELISAhelpstodetectantigenconcentrationinasample.

-Themicrotitrewellsarecoatedwiththeantigen.

-Antibodiesareincubatedinasolutionhavingtheantigen.

-The solution of the antigen-antibody complex is added to the microtitre wells. The well isthen washed to remove any unbound antibodies.

-Moretheconcentrationofantigeninthesample,lesserthefreeantibodiesavailable tointeract with the antigen, which is coated in the well.



AREASOFRESEARCHUSEDINICMR:

Bloodsamplesarecollected from the Animals and serum is separated and examined the Anitigen and Antibody Intaration byusing this ELISA technique.

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ANIMAL HEALTH MONIORING IN ICMR NARFBR

DEPARTMENT OF BIO SCIENCE &BIOTECHNOLOGY

For the award of the degree of

MASTER OF SCIENCE
IN
BIO TECHNOLOGY

Submitted by

MUPPIDI MAMATHA

M SC (BIO TECHNOLOGY)

Under the Guidance of:

Dr. L.SUSEELA

HEAD OF THE DEPARTMENT

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KRISHNA UNIVERSITY

MACHILIPATNAM-521001, A.P

JULY/AUGUST- 2023

DECLARATION

I hereby, declare that the subject matter embodied in this project report ,entitled **"ANIMAL HEALTH MONITORING IN ICMR NARFBR '' .**Which being submitted by me, for degree of the Master of Science in Bio Technology. Krishna University Machilipatnam,(A.P),India, is the result of research by me under the guidance of **Dr. L.SUSEELA** HEAD OF THE DEPARMENT, Department of Bio Science & Bio Technology . Krishna University, Machilipatnam.

Place: Machilipatnam

Date:

MUPPIDI MAMATHA

CERTIFICATE

This is to certify that the project report entitled "ANIMAL HEALTH MONITORIN G IN ICMR NARFBR" is a bonafied work carried out by Ms. M. MAMATHA (Y21BIT101014) under my supervision is submitted in partial fulfillment of the requirements for the award of degree of Master of Science Bio Science & Bio Technology to Krishna University Machilipatnam, A.P. No part of the dissertation has been submitted for any degree/diploma or any other academic award any where before.

PLACE: MACHILIPATNAM
DATE : 15/9/23

Dr. L.SUSEELA 15/9/23

(PROJECT ADVISOR)

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EXTERNAL EXAMINER

ACKNOWLEDGEMENT

When I present this project report on the completion Of my research work, I remember with gratitude the source of inspiration. Materials, assistance and guidance that lead to this achievement of goal.

I express my heartfelt gratitude to **Dr. L.SUSEELA** Head Of The Department, department of Bio Science & Bio Technology**, KRISHNA UNIVERSITY** Machilipatnam,A.P for his encouragement, constructive suggestions and constant inspiration throughout the entire course of study.

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MUPPIDI MAMATHA

Animal Health Monitoring – ICMR-NARFBR.

ABSTRACT

Laboratory animal experimentation in bio-medical research continue to remain crucial to find out better ways to understand , prevent, treat and cure diseases as currently there are no existing alternatives to substitute the biological systems. Worldwide, new drug research as well as tests meant for assuring the quality, safety, and efficacy of pharmaceutical products/vaccines/recombinant products involves experiments using animals. The use of animals for research is essential for the development of new and more effective methods for diagnosis and treatment of diseases that affect both human and animals.

Pathogens present in the environment are the biggest source of diseases and epidemics in the breeding and experimentation of laboratory animals. The ICMR NARFBR offers preclinical testing on laboratory animals (rodents), large animals (Sheep, Goat, pigs and Horse) and Non Human Primates under one roof. In fact, presence of microorganisms can critically influence the animal health status and reproducibility of experimental data. The ICLAS and WHO guidelines are formulated with aim of guarantee the best animal health state and valid support for research studies. In this preliminary study, health- monitoring program was carried out within the breeding of laboratory animals in ICMR - NARFBR facility.

The hygienic standards of ICMR have been developed over the last 100+ years. The key element of hygienic standardization is the monitoring of infectious agents that can compromise the animal's health, or dangerous for the personnel or interfere with the research. The health monitoring of laboratory rodents is essential for ensuring animal health and standardization in biomedical research. Progress in **Housing, Barrier system, zoonosis prevention and hygienic monitoring programs** led to enormous improvement of the microbiological quality of laboratory animals. The main viruses were analysed through molecular diagnostic techniques (PCR, RT-PCR). Bacteria were analysed through (STREAK PLATE METHODS, STAINING METHODS) and Enzyme immunoassays (ELISA-indirect). The established surveillance programme steadily guarantees animal health and ensure the most controlled

environmental and sanitary conditions. Further animal based research plays key role in pre-clinical studies and regulatory research in bio-tech, bio-pharmaceuticals and biomedical institutions across the country.

Keywords:

Health monitoring, Hygienic standardization, Molecular diagnostics. Rodent Pathogens. Research validity, Micro biome analysis.

INTRODUCTION

National Animal Resource Facility for Biomedical Research (NARFBR). The state government of Telangana offered 100 acres of land free of cost in the Genome valley, a biotech hub in Telangana. The Indian Council of Medical Research (ICMR), Department of Health Research, Ministry of Health and Family Welfare, Government of India has established National Animal Resource Facility for Biomedical Research (NARFBR) at the Genome Valley, Hyderabad .

The cabinet committee headed by the Honourable Prime Minister gave the approval for the formation of NARFBR on 18th November 2015. The Dept. Of Health Research, Ministry of Health and Family Welfare conveyed the orders on 3rd December, 2015 and subsequently, Indian Council of Medical Research(ICMR) issued a notification on 1st January, 2016 for the creation of NARFBR as a permanent institute under the aegis of ICMR.

ICMR- NARFBR has been established with the Vision to establish a state-of-the-art infrastructural facility for pre-clinical animal Experimentation of basic, applied, and regulatory research in the country. The institute offers preclinical testing on laboratory animal (rodents), large animals (Sheep, Goat, pigs and Horse) and Non Human Primates under one roof. India is ready to serve as per 'One Health Approach' and wholeheartedly committed for human welfare through Science as well as honours the Constitutional an moral obligations in order to ensure animal welfare.

In ICMR NARFBR when working with laboratory animals (mostly **Rodent Breeding** facility), scientists need to ensure that animals are not suffering from natural infections. Therefore, hygienic standards have been developed over the last 100+

years. The key element of hygienic standardization is the monitoring of infectious agents that can compromise the animal's health, are dangerous for the personnel or interfere with the research. However, scientists became aware that by eliminating such unwanted infectious agents the overall diversity of all microbes in research animals, the so-called **micro-biome**, has also been reduced. Moreover, it became clear that the micro-biome composition has an enormous impact on how research models react, e.g., to treatments. This might hinder the translation of findings in preclinical research to the clinical situation.

The health monitoring of laboratory rodents is essential for ensuring animal health and standardization in biomedical research. Progress in **Housing, Barrier system, zoonosis prevention and hygienic monitoring programs** led to enormous improvement of the microbiological quality of laboratory animals. While traditional health monitoring and pathogen detection methods still serve as powerful tools for the diagnostics of common animals.

REVIEW OF LITERATURE

As the most common research mammal in the world, mice are key to many aspects of biomedical research and in turn, scientific and medical advancements. Without mice, we would lack a crucial understanding of major diseases, including **cancer and genetic disorders like muscular dystrophy**, which has led to lasting benefits for humans and animals alike. It was only due to studies in mice and other animals. For example, we were able to develop vaccines for Covid-19, and at such a rapidrate.

By using mice, researchers can study how these diseases work in a living organism, and importantly, in one that is a good match for humans in many aspects. These principles then underpin the development and testing of new drugs, therapies and interventions. It is therefore not surprising to find that mice are the most used animal for scientific purposes in the EU, making up more than half (52.5%) of the total number of animals used in research – 5,459,433 animals in 2019.

• Few mammals have been as closely studied as the mouse. In this page we will explore some of the research areas in which mice have played a central role, as well as the discoveries and breakthroughs that have come about as a result, and theefforts in other countries to reduce, replace and refine their use**.**

Why are mice so important in biomedical research?

There are several important reasons why mice are so widely used in animal studies for biomedical research:

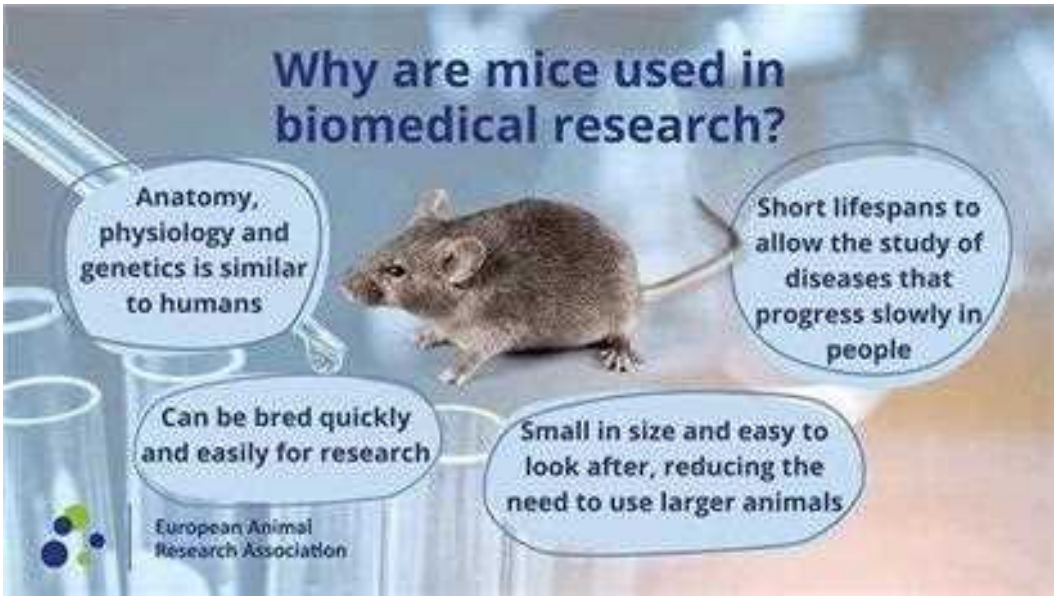
• Mice experience many of the **same diseases as humans** and have the same types of organs and bodily systems, which makes them and its genetic map (genome) hasbeen fully sequenced, which means that its genes can be switched on and off to study their effects.

• Mice have short lifespan (2-3 years), making them ideal for looking at **the progression of diseases** that may otherwise take years to develop and study inhumans.

• Mice are **quick and easy to breed**, and wean large litters, with a gestation period of around three weeks. This means that sufficient numbers of mice needed for research are available, which doesn't slow the pace of research – something particularly important for studying the effects of ageing, and in vaccine development for a new, or rapidly developing disease.

• Fruit flies and zebrafish are increasingly used in research, but if neither is a suitable model, the mouse is often the **first type of mammal** considered instead.

•Some scientists consider the differences between wild and laboratory mice to be so great that they think these laboratory animals could be classified as a **separate species**.



Which areas of biomedical research use mice?

Infectious diseases

Mice have been central to the study of many infectious diseases, including **influenza, hepatitis and Ebola** – all of which have the potential to be life-threatening. Where mice can be especially useful is in assessing how prone people are to certain infections and investigating why some do not develop immunity. While researchers can investigate these questions in human cells in the lab, these studies cannot

model the complex interactions that take place during an infection as accurately as in a living organism.

Researchers do conduct studies on humans, but not to the extent that is possible in mice. For instance, mice can be fed identical and tightly controlled diets, or be inbred to target the effect of different procedures on the same individuals – things that would be considered unacceptable using humans. It would also be too dangerous to test compounds and drugs on people without first knowing what the possible effects (and risks) would be, so animals such as mice are needed to fulfil this **crucial role in toxicity and safety testing**, in everything from experimental cancer drugs to vaccines.

Fighting Covid-19 – the vital role of mice

Covid-19 vaccines were developed at exceptional speed and studies using mice were vital in this process. Even though mice cannot catch Covid naturally, they can be infected with the SARS-CoV-2 virus, by creating genetically altered **'humanised' mice** (see box) that can then be used to examine the disease in detail. The genomes of these mice are altered so that they have a receptor for the ACE2 protein, found in humans, that allows SARS-Cov-2 to enter and infect cells.

Years before the Covid-19 pandemic, mice had already been used to gain important insights about other related diseases, such as SARS, which caused deadly widespread outbreaks in the early 2000s, and MERS, which first emerged in 2012. It was thanks to these earlier studies that researchers could study how the SARS-CoV-2 virus infected human cells, without needing to start from scratch with a new mouse model that would have inevitably slowed the pace of Covid-19 research.

For example, in research at the University of Iowa, USA, back in 2007, scientists used humanised mice expressing ACE2 to identify a path to lethal infection by the coronavirus that causes SARS, helping to understand how the disease develops to devise new treatments.

Humanised mice for Covid-19 allow researchers to answer **key questions about the virus** itself, such as how it begins to infect organisms and is transmitted from one body to another, how an infected host's immune response reacts, as well as the short- and long-term effect of various treatments and experimental vaccines. All of these aspects are needed to shed light on how the disease works in order to develop effective drugs and measures against it.

Credit: University Medicine at Johannes Gutenberg University of Mainz

HUMANISED MICE

Mice and humans are genetically very similar, with almost all mouse genes sharing the same functions as our genes. But there are still limits to how much we can compare ourselves to mice, especially when it comes to research. That is why large numbers of mice used in biomedical research are genetically altered (GA) to **mirror more human-like traits** and biology, typically at the level of cells or genes.

This 'humanising' process is done by inserting something like a fragment of human DNA or a tumour into them, so that the mice react in a similar way to a human. Humanised mice therefore allow researchers to **explore far more human genes, proteins and processes** than would otherwise be possible.

Because of the current technical limitations of gene editing, not every GA animal that is bred for this purpose can be guaranteed to display the genetic mutations or characteristics that were intended for a particular type of research. It means therefore that they are bred but not used in research. However, the actual breeding ofa GA mouse is counted as a research procedure and is recorded – these mice came to a total of around 12.5 million animals in 2017 (when they were last recorded).

The 'bred but not used' process is currently being refined through the use of powerful and accurate gene editing tools, such as CRISPR, that can directly edit mouse embryos to reduce the number of animals that are needed. You can read more about the numerous diseases that CRISPR mice have used to study in this Review, How animal studies play their role in a biomedical research revolution.

Cancer

In the last decade, there has been a significant improvement in the survival rates of many types of cancer, and studies in mice have been among the **most valuable tools used**.

A major advance is the first approved treatment for breast cancer – the **monoclonal antibody, Herceptin** – which was only developed due to initial research in mice and other animals such as rats and hamsters. These studies laid the foundations for the discovery and understanding of the HER2 protein, which can be targeted to reduce tumour growth. Translated into the clinic, studies of breast cancer patients has shown that adding Herceptin to chemotherapy improves survival and keeps cancer at bay for longer compared to chemotherapy alone.

Some of the cancer breakthroughs that have resulted from research in mice include the development of a type of **immunotherapy cancer treatment**, which uses the body's immune system to attack and kill cancer cells (called immune checkpoint inhibitors). The researchers who pioneered this treatment, James Allison and Tasuku Honjo, won the 2018 Nobel Prize in Physiology or Medicine.

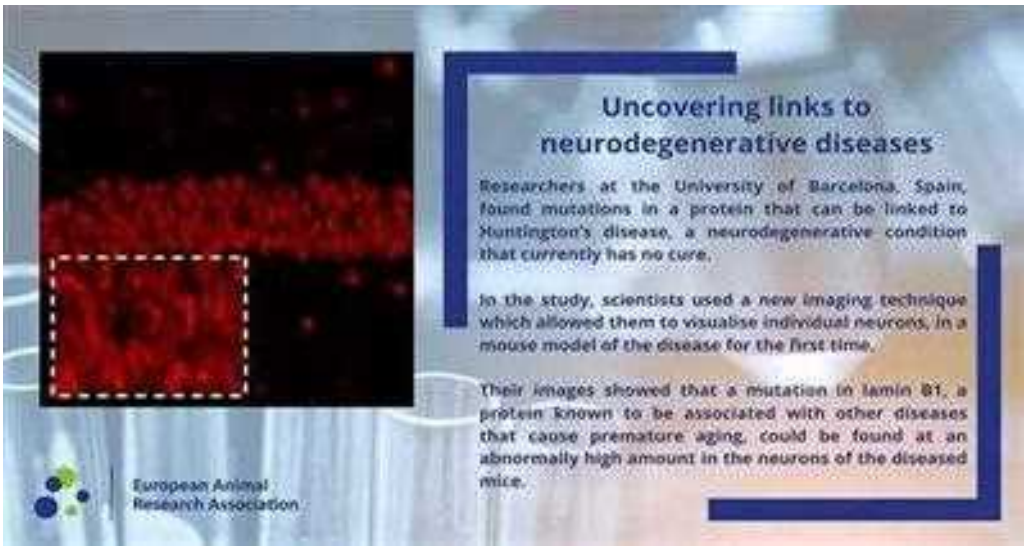
Meanwhile, ongoing cancer research is using mice to help in a broad range of areas, for instance research at EPFL in Lausanne, Switzerland, has revealed a new way to stop **lung cancer tumours** from becoming resistant to treatment. And the University of Bristol, UK, used mice to show that a cancer drug can be repurposed to help with heart attack recovery.

Alzheimer's disease and dementia

Mice can provide important insights into the **mechanisms of Alzheimer's**, the most common type of dementia, for example by showing hallmarks of the disease, suchas the loss of neurons in the brain, or symptoms like memory loss, as investigated by institutions including the Institute of Biomedicine of Seville, Spain. Our understanding of other neurodegenerative conditions, such as **Huntingdon's disease** (see box below), have also benefited from the use of mice.

Mice not only allow researchers to explore new treatment options, but also to assess the **potential success of drugs and compounds** before they are trialled in humans – so that they don't cause harm or work differently to how we expect them to.

However, mice are not always the best models for the human brain, especially when it comes to biology. Monkeys are usually used instead as their brains more closely resemble ours and share many of the same features.

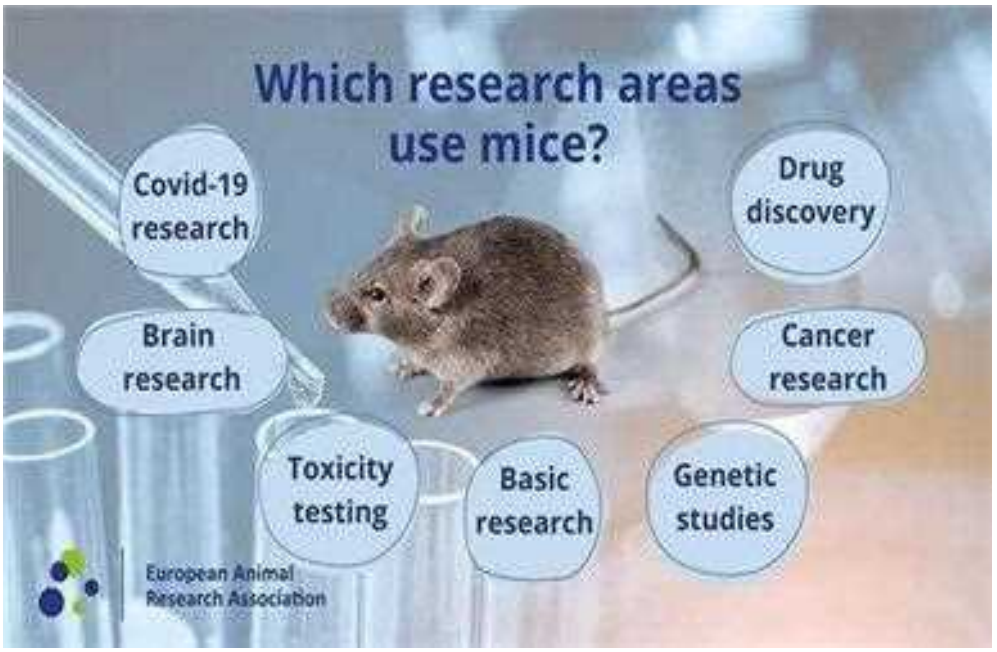


Other diseases and conditions

Research into a range of major conditions, such as **asthma**, **depression**, **lung conditions** **and** **obesity**, have involved the use of mice. Mice have also been used to understand **health phenomena**, including one of the biggest global threats, **antibiotic resistance** – for example in a 2022 study by EARA member the Luxembourg Center for Systems Biomedicine – and the effects of zero gravity in space.

Basic research

One of the main areas where mice are used is in studies done to gain a **fundamental understanding of the body**. This includes the functions of different genes and proteins, the differences between the states of health and disease, anatomy and physiology, and how biological invaders such as viruses cause infection and impact our health. Researchers can then take this information and begin to **apply it to humans** to provide insights into how we grow, age or become ill. A study includingthe Max Planck Institute of Molecular Cell Biology and Genetics, Germany, showed how genes work to influence the **development of diabetes** in mice, for example.



Are mice being replaced or used in smaller numbers?

Under EU Directive 2010/63, researchers must use **non-animal alternative methods** whenever they are possible, with the ultimate goal of replacing animals in research entirely. Research animals in the EU can also only be used if there are **no suitable alternatives**. The 3Rs principle (Replace, Reduce, Refine) is an ethical framework for minimising, or avoiding the use of animals in research and is a standard practice in research around the world.

New approach methodologies (NAMs), such as studying cells in the lab (in vitro), lab-grown mini organs (organoids) and natural or engineered tissues grown inside computer chips (organs-on-chips), which mimic human systems and physiology, are emerging as alternatives to animal studies. When these non-animal methods are not possible, the use of mice can also be reduced by replacing them with other animals, such as zebrafish or fruit flies.

There are moves towards **reducing the numbers of GA mice**, for example by freezing the eggs, sperm or embryos from GA mouse lines, so that they can be accessed at a later date. There is also increased sharing of these strains of mice between researchers, meaning that fewer are ultimately used in the long term.

Credit: University Medicine at Johannes Gutenberg University of Mainz

Is research on mice and rats the same thing?
Rats are also a very common animal used in biomedical research alongside mice,but even though the two might seem similar, there are some important differences when it comes to what they bring to research. Mice and rats have different cognitive and social behaviours, and **react differently to stress, handling, and certain drugs and substances**, all of which mean that one is more suitable over the other, depending on the type of research. Rats are more intelligent than mice, for example, making them better models for conditions like addiction.

The Laboratory Rat as an Animal Model for Osteoporosis Research

Osteoporosis is an important systemic disorder, affecting mainly Caucasian women, with a diverse and multifactorial etiology. A large variety of animal species, including rodents, rabbits, dogs, and primates, have been used as animal models in osteoporosis research. Among these, the laboratory rat is the preferred animal for most researchers. Its skeleton has been studied extensively, and although there are several limitations to its similarity to the human condition, these can be overcome through detailed knowledge of its specific traits or with certain techniques. The rat has been used in many experimental protocols leading to bone loss, including hormonal interventions (ovariectomy, orchidectomy, hypophysectomy, parathyroidectomy), immobilization, and dietary manipulations.

The aim of the current review is not only to present the ovariectomized rat and its advantages as an appropriate model for the research of osteoporosis, but also to provide information about the most relevant age and bone site selection according to the goals of each experimental protocol. In addition, several methods of bonemass evaluation are assessed, such as biochemical markers,densitometry,

histomorphometry, and bone mechanical testing, that are used for monitoring and evaluation of this animal model **in preventive or therapeutic strategies for osteoporosis**.

Osteoporosis is a multifactorial skeletal disease, characterized by reduction in bone mass and disruption of the micro architectural structure of bone tissue, resulting in loss of mechanical strength and increased risk of fracture.

The disorder can be localized or involve the entire skeleton. Generalized osteoporosis can be primary (postmenopausal and senile) or secondary. In the European Union, osteoporosis is a leading cause of mortality and morbidity in the elderly and a key factor in the high cost of medical care.

Although osteoporosis usually makes its appearance late in life, and age is a major risk factor, its roots can be tracked back into adolescence. Particularly during periods of rapid bone growth, dietary calcium levels are of high importance.

Other factors that contribute to the pathogenesis of osteoporosis are lifestyle and

genetic and hormonal attributes. Reduced physical activity increases the rate of bone loss, and muscle contraction is the prevailing source of skeletal loading. Regarding hormonal factors,women, especially in the decade after menopause, can show a severe reduction of bone mass, thus explaining the high incidence of osteoporotic fractures in women compared with men.

The multiple factors implicated in osteoporosis, its obscure pathogenesis, the dramatic decline in quality of life, high incidence of the disorder (especially in postmenopausal women),financial cost, and high mortality, make the need for further experimentation in animal models imperative.

Experimental research can improve our understanding of pathogenesis and of the activity of pharmaceutical agents in the prevention or treatment of the disease. Although many aspects of the disorder have been revealed, others remain unclear, including the mechanisms involved in calcium homeostasis in the extracellular space and its effect on bone physiology and disease and the cell and molecular pathways triggered after mechanical loading to orchestrate bone renewal.

Current research is focused on new therapeutic possibilities targeting the osteolytic enzymes of the osteoclast and the mechanisms activating bone progenitor cells and those controlling apoptosis as new potential treatments.

Many therapeutic advances in the management of osteoporosis were studied first in diverse animal models and then entered clinical practice.

All of these models should fulfill similar basic criteria: they must comply with national and local ethical and legislative considerations, be accessible to experimental centers, be easy and safe to handle, have a low cost of acquisition, require little maintenance, reliably reproduce the disease and the biological material to be examined should be readily available.

Laboratory rats meet most of these criteria. In addition, the availability of detailed knowledge of the rat skeleton and protocols for rapid induction of osteopenia, have increased this model's popularity.

Here we review the advantages and limitations of the use of the laboratory rat in osteoporosis research.

COMMON LABORATORY ANIMALS –IN ICMR NARFBR

Nowadays, widely used hygienic monitoring programs and sanitation procedures lead to enormous improvement of the microbiological quality of laboratory animals, producing breeding colonies, which are free of pathogens and even free of most opportunistic pathogens.

Undoubtedly, modern **Rodent research management** has been improved and enabled the breeding of Immuno-compromised animal models without microbial induced diseases.

Physiologically and anatomically there are similarities between the humans and animals at organs and organ systems, which function in the similar fashion.

This similarity makes animal ideal for the study and development of products and techniques of humans.

By using laboratory animals various discoveries have been made such as diphtheria and polio vaccine, insulin for the treatment of diabeties mellitus, heart valve replacement, antibiotic therapy, manic depressive drugs, etc

CLASSIFICATION OF LABORATORY EXPERIMENTAL ANIMALS IN RODENT BREEDING FACILITY

RODENTS

NON-RODENTS

MISCILLANEOU

SRODENTS:

Rodents are mammals

Characterized by **upper and lower pairs of ever-growing rootless incisor teeth.**

Largest group of mammals –approx(4660-species)

Most commonly used: **Mouse, Rats**, Guinea pig, Gebril , Hamster ,etc.

RODENT BREEDING FACILITY

Dedicated for breeding of SPF quality rodents and lagomorphs such as rats, mice, rabbits, hamsters and Guinea pigs.

This is Class 1,00,000/ISO 8 facility with controlled environmental conditions (temperature : 22 ± 20C and 40-70% humidity).

In ICMR Rats, mice and rabbits are used for Research.

Facility has dedicated labs for **health monitoring, genetic monitoring, preclinicaland vaccine testing services.**

Laboratory Rodents

Mouse: BALB/C, C57BL/6 (Black) , SWISS ALBINO

Rat: Wister and Sprague Dawley

NON –RODENTS

GUINEA PIG: Dawkins heartly

Rabbit: Newzland White (NZW)

Breeding Methods

1. Inbreeding – close relation
2. Out breeding
3. Random breeding
4. Selective breeding

SWISS is the part of BALB/C and it is outbreed or random breed.

BALB/C and C57BL/6 are inbreed.**Chromosome number**:

1. Rats: 48
2. Mice: 20
3. Rabbits: 44
4. Guinea pig: 64

Gestation period:

Mice (C57bl6):18.5 days

Gestation lab rats: 21-23 days

Litter size:

C57BL/6 (more than 10)

BALB/C (8 -14)

SWISS (15 to16)**Caging**

Systems:

1. Barrier System
2. Convention System
3. Individual Ventilation Caging System

Weaning Period:

21 days in mouse

15 days in guinea pig

3 months in rabbits

Mating Methods:

1. Monogamous
2. Polygamous
3. Mass Mating
4. Hand Method Mating
5. Harem Mating

Bedding Materials For Animals:

Paddy Husk , Corn Cobb , Wodden Chips ,Cellulose Membrane , Raw Paper

TYPES OF EXPERIMENTAL ANIMALS USED IN LABORATORY

Animals mostly used in **RODENT BREEDING /EXPERIMENTATION** facility-ICMR NARFBR

1. MOUSE
2. RAT

MOUSE: (MUS MUSCULUS)

The most **common mammal** among laboratory animals - **MICE**

CHARACTERSTICS

1. Mice are small laboratory animals weighing about (18-30g).
2. They are very sensitive and consume small doses of drugs.

3. They can be easily handled.
4. Drugs are injected through intraperitoneally or intravenously into one of their superficial tail veins.

AREAS OF RESEARCH

The following are the research areas where the above mentioned animals are used as experimental animals.

1. In cancer and genetic research.
2. In acute toxicity studies
3. In screening of various drug activities specially central nervous system (CNS) activity.

MICE SEXING: (DISCRIMINATE SEX- MALE AND FEMALE

The distance between the anal and genital orifices is greater in –male compared to –female.

HANDLING AND RESRAINT OF MICE

Gently but firmly

Wear disposal gloves

Wash your hands prior to and after handling

Wear a clean laboratory coat.

GENTICALLY MODIFIED MICE

Knockout mouse

Knock in mouse Knock out mouse

It is genetically engineered mouse in which researchers have INACTIVATED or KNOCKED OUT , an existing gene. This is the most important animal model to **studythe role of genes that have been sequenced but whose functions have not been determined.**

This causes changes in mouse phenotype(appearance, behaviour ,physical and biochemical charecteristics)

Causing a specific gene inactivation, and observing any differences from normal behaviour and physiology, we can infer its probable function.

May also be a useful experimental pathological model to test the efficacy of new drug.

RAT: (RATTUS NOVERGICUS)

Laboratory rats play an important role in biomedical research across range of therauptic areas.

These are closely related to the human genome.

CHARACTERISTICS

1. Rats have a larger weight than mice(about 300-500g)
2. They can withstand long periods of experimentation under anaesthesia.
3. They can be easily handled if treated kindly.
4. Drugs can be injected through Intravenously(I.V) into their tail veins. Subcutaneously(S.C) , intramuscularly(I.M)
5. They can also be given drugs orally by means of stomach.

PROPER METHOD OF HANDLING:

To initially restrain a rat, the handler should be gently grasp it around the shoulders.

The handlers thumb can then be placed under the rats mandible to prevent bites, and the rats hind limb scan can be supported with the other hand.

Restraint should be firm but not too tight as this will impede the animal's respiration.

Gastric gavage orally in rats.

Used in research:

Mostly used rat-(ALBINO WISTAR RAT)

1. To study behavorial and psychological studies .
2. Cardiovascular studies.
3. Neural regeneration.
4. Space motion sickness.

5. Used in toxicology and pharmacology studies.

STANDARD OPERATING PROTOCOL FOR LABORATORY RODENTS USED INRESEARCH

1.1 **Introduction**

The intent of this Standard Operating Protocol (SOP) is to describe acceptable methods of transport, housing, care, enrichment, anesthesia, and euthanasia of rats and mice.

This SOP is intended for personnel that use rodents in research , who care for animals in the Animal Care Facility.

This procedure is approved by the UWSP Institutional Animal Care and Use Committee (IACUC; October 31, 2011)

1.2 **Requirements of personnel implementing this SOP**

The principal investigator or lead instructor must ensure that all individuals responsible for rodent care and/or use are familiar with all elements of this SOP as well as additional requirements outlined in individual protocol.

1.3 **Safety**

Personnel should be aware of safety equipment locations and recommendations for use. General safety protocols can be found on the UW-SP Environmental Health and Safety website: http://www4.uwsp.edu/ehs/

No food or drink intended for human consumption is allowed in the facility,except in the administration office.

If any maintenance issues arise such as extreme changes in temperature orroom flooding, maintenance can be reached. In case of emergency, personnel should call Campus Security .

The Animal Care Facility maintains restricted locked access. The surgery room and all rooms containing rodents must be locked before personnel leave the facility. Only authorized personnel are admitted into the facility. Visitors must be supervised at all times.

1.4 **Transportation**

Personnel transporting rodents, whether intra- or inter-institutionally, must ensure the safety and comfort of the animal(s) at all times.

Transportation media must provide adequate food, water, temperature, humidity, comfort, and overcrowding must not occur

Delivery of animals must be coordinated to occur during business hours.

1.5 Rodent Inventory

Rats and mice are to be housed in separate rooms. Identification cards must include source of animals, date of arrival, date of birth, species, sex, name or other identification.

If any Rats should be housed two per cage unless protocol specifies otherwise.

If a cage with two large rats is becoming soiled in less than a week, or contains animals who are causing each other harm, then those animals should be separated.

Mice, baby/juvenile rats may be housed more than two per cage. Births and deaths should be recorded on daily log sheets.

1.6 Husbandry

All personnel should have working knowledge of cleaning, feeding, watering, andenrichment schedules

Only trained workers are authorized to implement these tasks.

Log sheets must be completed daily for each room that houses rodents.

Personnel must ensure that appropriately sized cages for each animal are used to prevent escape.

1.7. a. Observation & monitoring

Each age must be checked daily for water and food levels, animal health, and animal births or deaths.

Log sheets must be completed daily.

The acceptable temperature range for rats and mice is between 68 and 79degrees Fahrenheit (20-26 degree Celsius)

Relative humidity should be between 30% and 70%.

1.7.b .Feeding

All rodents should have adequate food in their food hopper (at least ½ cup).

Food levels must be checked daily, and more food should be added if levels are observed to be low.

A closed food container should be available in each rodent room.

If food containers are empty, personnel may obtain more food from the supply room. The date that the container was refilled should be logged on the food container.

Recommendation from the feed manufacturer should be followed regarding shelf life and storage.

Additional food may be provided to rodents as 'treats' at the caretaker's discretion.

If this food is stored in the Animal Care Facility, it must be labelled "not for human consumption" and dated.

1.7. c Mortality & removal

Mortalities should be removed daily from each cage, recorded with the daily log and on the Euthanisia/ Natural Death log sheet, placed in sealable bags, labelled with the date that animal was observed to be dead, and placed in the freezer.

Rodents that are safe for avian or reptile consumption may be donated for that Purpose.

All other animals are placed in a cremation bag and delivered to the Biology stockroom.

1.7. d Cage cleaning

Cages are cleaned at least once per week. Cleaning consists of replacing cage, bedding, water bottle, sipper, and any toys or housing inside cage.

The wire cage tops must be sanitized at least once per month .

Dirty housing equipment, water bottles, sippers, and toys are sanitized in the cage washer.

Before cages are placed in the cage washer they must be rinsed and scrubbed out, using soap if very dirty.

If newspaper is used in place of bedding in an extra-large cage, it must be replaced before rotating animals in that cage, and the pan must be disinfected.

After bedding is disposed of in waste container, waste should be emptied in the loading dock dumpster.

1.7. e Room and facility cleaning

Floors in the Animal Care Facility should remain generally free of bedding and other debris.

Food spills must be cleaned up immediately. Regular facility cleaning is scheduled as follows:

1. Floors should be swept and mopped weekly at a minimum, and more often as needed.
2. Cage racks should be sanitized once per month, and swept as needed.
3. All areas should be kept free of clutter. Vent filters in rooms containing rodents should be changed every two months, and those in all other rooms should be changed biannually.

1.7.f Disease control

Personnel handling rodents or any associated equipment should wash their hands thoroughly upon completion of work or more often as needed.

All housing, feeding, and watering equipment must be disinfected before used for a different animal. Food and water that has been used for one animal (or set of animals) must not be given to another animal.

Personnel should ensure that no open wounds are present on their hands before handling the animals.

Disposable gloves are available for use in the cage wash room.

1.7 **.g. Quarantine**

Rodents suspected of having a contagious disease should be separated from other animals. Quarantine areas should be managed according to rigorous infectiousagent control practices.

1.8 **Enrichment**

Enrichment is highly encouraged and should be provided as often as possible. Some protocol may specify that certain animals are to receive no enrichment.

The following are recommendations for enrichment:

Two mice per cage

Housing structures inside cages, which could include cardboard boxes, PVC elbows, jars, etc.

Wheels

Fresh herbs for scent stimulus

Rotation of rats to large cage (rats from more than one cage should not be mixed in the large cage)

Treats: peanut butter, bird seed mixed in (clean) bedding, fruit, vegetables, bread, cereal, etc. Only asmall amount of perishable food such as fruit should be place in a cage at one time.

Plastic bottles with food inside or 'congs' containing food (they have to work to get it out)

Mice should be provided with nesting squares

Rats should be handled on a regular basis

1.9 Anesthesia

Anesthesia should be used before performing procedures that may cause more than momentary mild discomfort.

Carbon dioxide and isoflurine are approved for anesthesia.

Personnel should adhere to procedures outlined in applicable protocol

1.10 Euthanasia

Euthanasia of rats and mice are as follows:

Carbon dioxide halothane, isoflurane, sevoflurane and Desflurane, with or without nitrous oxide

Regardless of method used, euthanasia must be performed in the provided container under the fume hood in the surgery room.

Personnel performing euthanasia shall perform checks to determine whether the animal is expired while the animal remains in the chamber.

Personnel performing euthanasia shall fill out the Euthanasia log.

Any personnel performing euthanasia must receive appropriate training to perform the

Procedure, adhere to IACUC-approved protocols and institutional policies.

1.11. Disposal of carcasses

Upon death, all animals should be placed in the freezer. Those that are safe for animal consumption may be donated and those that are not must be delivered to the stock room in a cremation bag for incineration.

INTRAVENOUS ADMINISTRATION IN RODENTS:

The most accessible vessels for intravenous administration in rodents are veins that run the l lateral aspects of the tail.

Contact the IACUC office for one-on-one training sessions.

Supplies:

Sterile 27-30 gauge needles for mice

Sterile 25-27 gauge needles for rats

500uL to 1ml syringe for mice is recommend

1ml or 3ml syringes for rats

Heating device

Gauze sponges

Isoflurane, Anesthesia System (recommended)

Note: It is highly recommended that new users of this technique anesthetize the animals while c procedure until proficiency is obtained.

Procedure Steps:

Weigh each animal before injection

Up to1% of the animals body weight in the volume can be administered per injection.

1. Record body weights and agent volumes to be administered for each animal.

2. Prior to injection, warm animal for 5-10 minutes to dilate the veins. Animal may be warmed by placing the animal in a commercially available warming box, brass restrain or by using a warm water circulating pad placed under the cage. These are the safest and most effective ways to warm rodents. If an overhead heat lamp is used, extra care must be taken to prevent overheating the animal.

3. It is recommended to lightly anesthetize the animal.

4. When anesthesia is necessary, position the animal on its side on a rodent safe heat source or circulating warm water pad to maintain thermoregulation during anesthesia.

5. Conscious animals need to be restrained using a commercially available restraint device of appropriate size (see below). A pre warmed brass restraint provides heat for vasodilation, reducing the need for pre warming mice in a cage. The duration of

the restraint should be kept to a minimum, and the equipment washed frequently to prevent pheromone-induced stress or cross contamination. Rodents sometimes spin in the restrainer; be sure to confirm to orientation and location of the lateral tail vein before performing injections.

6. Hold the syringe with the dominant hand near the bottom so that the remaining fingers are near the plunger and can easily push the agent into the vessel without disrupting the needle in the vein.

7. Syringes should be prepped with no air bubbles. One syringe and needle are recommended per mouse/rat. Needles should be sharp and replaced after two attempts. Insert the needle (small gauge, 27-30 for mice and 25-27 for rats), bevel up, into the vein towards the direction of the head. Keep the needle and syringe parallel to the tail. Aspiration is not advised as it may cause the vein to collapse, but a flash of blood in the hub of the needle may be seen when first placed. Proper placement may not be verifiable until injection occurs, but when placed correctly the needle should advance smoothly into the vein.

8. Slowly inject. If there is resistance and/or a blister or white area appears above the needle on the tail, the needle should be removed and re-inserted above the first site.

9. Remove the needle and apply gentle compression until bleeding has stopped.

10. If the animal was anesthetized, monitor the animal during the recovery process.

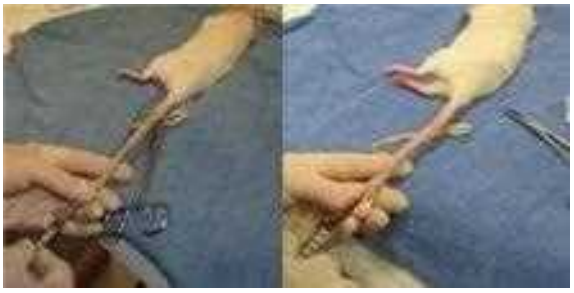
11. Return animals to their cage and observe to make sure that bleeding has not resumed. **Note:** With brown or black mice and rats, an additional light source may be necessary to aid in visualizing the tail veins. Rats have scales making the vein difficult to see, especially in older adults. The scales are removed by gently cleaning the tail with a saline or chlorhexidine solution making the veins more apparent- wipe in the direction of in the direction of the scales to avoid irritation to the tail.

Mouse:

Grasp the tail at mid-length or at the distal (further down the tail) end. The index and middle fingers of the non-dominant hand are placed around the tail above where the needle will be inserted (digital pressure will act as a tourniquet). The lower part of the tail is held between the thumb and ring finger below the injection site. Put slight tension on the tail by applying pressure with both sets of fingers. Needle should enter the vein at a shallow depth, keeping syringe and needle parallel to tail. Release pressure to the proximal fingers before administering the agent into the vein. No resistance should be felt when depressing the plunger.

Note: With mice, elevating the animal about 4-6 inches off the table may be helpful with keeping the needle and syringe parallel to the vein.

Rats: A tourniquet is used to constrict the vein to allow visualization and access to the vein for injection mid-length or at the distal (further down the tail) end. A tourniquet is made with a rubber band wrapped around the top of the tail and held together firmly with a hemostat. The tourniquet is released before the agent is administered into the vein.



Agents:

This procedure recommends anesthesia. All agents administered to animals should be listed in the "Agents" section of the RIO IACUC protocol.

Adverse Effects:

Adverse effects should be listed in the “Adverse Effects” section of the RIO IACUC protocol. Examples of potential adverse effects include: Peri-vascular irritation, Blood loss .

LAB ANIMAL HEALTH MONITORING

Monitoring the health of laboratory animals is one of the many responsibilities researchers bear when performing their critical research.

Our portfolio of lab animal health monitoring services, including pathology, serology, PCR infectious agent testing, and more, can help you detect and eliminate any pathogens that could negatively influence your studies.

Our protocols in lab health monitoring include

1. pathology
2. serology
3. microbiology
4. parasitology
5. pcr infectious agent testing
6. immunoassays-(ELISA)

To effectively detect pathogens, it's best to use multiple lab animal health monitoring testing modalities at various levels within a research facility.

We are always looking to identify and incorporate the most analytical sensitive and specific technologies in order to provide you with the most accurate reproducible results.

MATERIALS AND PROCEDURES OF LABORATORY ANIMALS:

1. **Streak plate method**
2. **Gram staining**
3. **Preparation of various media**
4. **Polymerase chain reaction**
5. **Elisa**

STREAK PLATE METHOD

INTRODUCTION: Streaking is a technique used in microbiology for the isolation ofsingle colonies of microorganisms, either from a mixed species or from the same species. This technique is mostly applicable to bacteria but is also used for some yeasts. It is an old technique that has been in use since the time of Rober Koch. It was first demonstrated by Loffler and Gaffky in Koch's laboratory.

PRINCIPLE : The streak plate method is based on the principle of dilution. It can be described as a rapid qualitative isolation technique. The main criterion of isolation is to obtain a reduced number of colonies. In this technique, a loopful of culture spreadon an agar plate to get individual cells far apart enough from each other. The streaking method gradually dilutes the inoculum such that the bacterial cells can be

counted as colony forming units (CFUs).

MATERIALS REQURIED :

Petri dish — sterile , disposable, 90 mm diameter.

Media — e.g. f2.

Agar — Bacto -Agar , Difco .

Wire loops — nichcrome or platinum.

Bunsen burner or small flame.

Parafilm.

Types of Streak Plate Method:

Quadrant Streaking: This is the most common method of streaking, where the petri dish is divided into four quadrants and then inoculated. It is also known as a four-quadrant streak. A loopful of inoculum is taken and streaked such that the first quadrant contains the highest concentration of the inoculum, followed by the second quadrant, third quadrant, and fourth quadrant. This is a discontinuous method where the loop is sterilised after streaking in every quadrant. By the time the fourth quadrant is streaked, the inoculum is diluted enough to give rise to individual colonies.

T-Streaking: In this method, the petri dish is divided into three quadrants by drawing the letter 'T'. Similar to four-quadrant streaking, it is a discontinuous method where each quadrant is streaked after loop sterilisation, and the quadrant that is streaked last gives isolated colonies.

Continuous Streaking: In this type of streaking, the inoculum is spread from one edge to the centre of the plate. The plate is rotated 180°, and the remaining portion is streaked without sterilizing the loop. Another form of continuous streaking is used for diagnostic purposes. The petri dish is divided into sections, and different cultures, such as urine, sputum, pus, etc., are streaked in each section to get maximum output.

Radiant Streaking: In this streaking method, the inoculum is spread on one edge of

the petri dish. From the edge, vertical lines are streaked in the upward direction. Next, the vertical lines are streaked horizontally to obtain pure isolated cultures.

Streak Plate Method Procedure:

-Sterilise all the instruments, flasks and media that are required for the streaking procedure.

-Clean your work area using a disinfectant to minimise any contamination.

-Set up the bunsen burner in your work area carefully.

-Wash your hands with an antiseptic solution before handling any microbial solution.

-Label the petri dish with all important information, such as your name, date, media used and the culture being inoculated.

-To pick up the sample, you can use either a metal loop or disposable plastic loops.

-A loopful of sample is streaked on the first quadrant in a back-and-forth motion on the

-Sterilise the loop by heating it in the bunsen burner if using a metal loop.

-Streak the other three quadrants by a similar method.

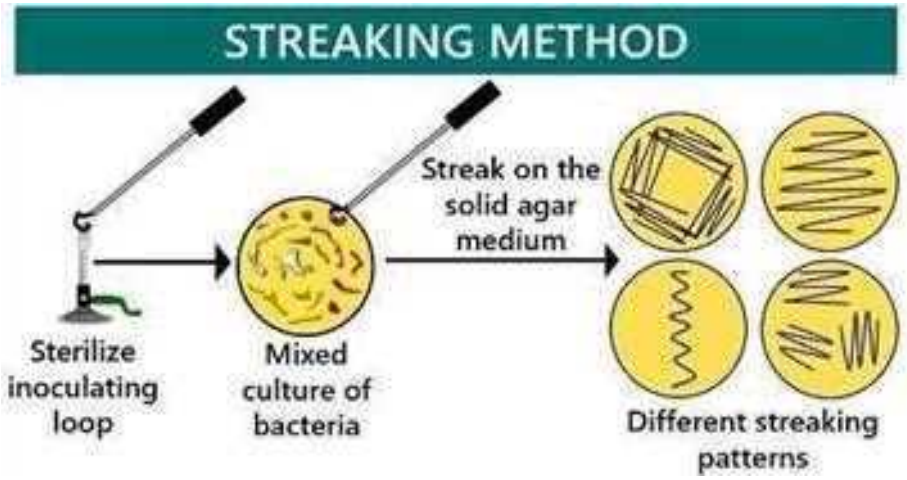
-Close the lid of the plate after streaking, and store the dish upside down in an incubator with optimal temperature.

Advantages of the Streak Plate Method:

It is one of the most popular techniques used to obtain isolated colonies of bacteria.

It finds a great application in biotechnology as it can be used to identify transformed bacteria from non-transformed bacteria by adding an antibiotic to the growth medium.

It also finds great application in diagnostic purposes.



STREAK PLATE METHOD USED IN ICMR

- To check the presence of different types of micro organisms inside and outside the body of animals which are grouped under Rodents, Small Ruminants ,Canines ,Primates and Equines.

GRAM'S STAINING

INTRODUCTION: Staining, in microbiology, can be defined as a technique which is used to enhance and contrast a biological specimen at the microscopic level. Stains and dyes are used to highlight the specimen at the microscopic level to study it at higher magnification for histopathological studies and diagnostic purposes.

However, staining is not just limited to biological specimens, it can also be used to study the structure of crystalline polymers.

Preparation of the Biological Specimen:

The preparation of the biological specimen to be analysed under a microscope depends on the type of staining. Given below are some procedures that are carried out.

Wet Mounting: Living biological specimens are mounted on a glass slide with

water and specific stains.

Fixation: It is a multi-step process which is done to preserve the shape of cells and tissues. Heat fixation is done to kill and adhere the specimens. Chemical fixation is done to generate strong bonds and increase the rigidity of the samples. Common chemical fixatives used are formaldehyde, picric acid, methanol and ethanol.

Mordant: Mordants are chemical agents that are used along with dye to make the specimen stainable, which otherwise is unstainable. Mordants are of two types:

Basic Mordants: They react with acidic

dyes.**Acidic Mordants**: They reactwith basic

dyes.

When staining is done with the help of mordants, it is known as indirect staining. On the other hand, when staining is done without the help of mordants, it is known as direct staining.

Permeabilisation: This procedure involves treating thespecimen with a surfactant that dissolves the cell membrane allowing easy staining with the dye.

Types of Staining Techniques

Gram's staining: This staining procedure is used to identify bacteria based on their cell wall composition. There are two types of Gram's staining, and the bacteria can be divided into gram positive or gram negative bacteria. It uses crystal violet for the staining of cell walls, iodine as the mordant and safranin or fuchsin as the counterstain

Biological Stains

Some of the most commonly used biological stains are listed below:

-Acridine orange :It is a fluorescent cationic dye that is selective to nucleic acids. It is used during the cell cycle to analyse DNA molecules.

-Coomassie blue: It is used in gel electrophoresis to stain the proteins blue.

-Crystal violet: It is used in Gram's staining along with iodine to stain the bacterial cell wall in purple colour.

-Eosin: It is used as a counterstain to hematoxylin that imparts a red colour to the cytoplasm and its components.

-Ethidium bromide: It provides a red-orange fluorescent stain to the DNA after intercalating with the molecule.

-**Iodine**: It is used as a mordant in Gram's staining.

-**Malachite green**: It gives a blue-green colour when used as a counterstain against safranin that is used in endospore staining.

-**Methylene blue**: It is used to stain animal cells because it enhances the nuclei.

-**Safranin**: It is a red cationic dye that is used as a counterstain in both Gram's staining and endospore staining.

AREAS OF RESEARCH IN ICMR:

Different samples of animals such as feces , hair, and skin scrap are send to the microbiology lab for testing the presence of microbes. After the plating technique the grown microbes are examined by using this staining technique . We found **SALMONELLA TYPHI** which is a gram negative bacteria observed in pink colour in the above slide.

PREPARATION OF VARIOUS MEDIA

Preparation of Mac Conkey Agar:

Purpose: Mac Conkey agar is a differential medium to distinguish lactose fermenting gram negative bacteria from non lactose fermentors ; lactose fermentors arecoloured pink, while non lactose fermentors are pale. It is appropriate for stool, urine, urogenital specimens, throat swabs, ear and eye swabs, wound swabs, sputum, aspirates, cerebrospinal fluid.

Procedure for preparation:

-Suspend 51.1g of powder in 1 litre of distilled or deionized water.

-Heat until completely dissolved.

-Sterilize in autoclaved at 121 C for 15 minutes.

-Cool to 45-50 C.

-Pour 15-20 ml of the ready media on to petri dishes.

-Leave standing for thirty minutes to solidify.

-Perform sterility testing as described in Section 7.

-Label the bottom of each plate with date of preparation and batch number.

-Store the culture media plates upside down at 2-80C sealed in plastic bags to reduce chances

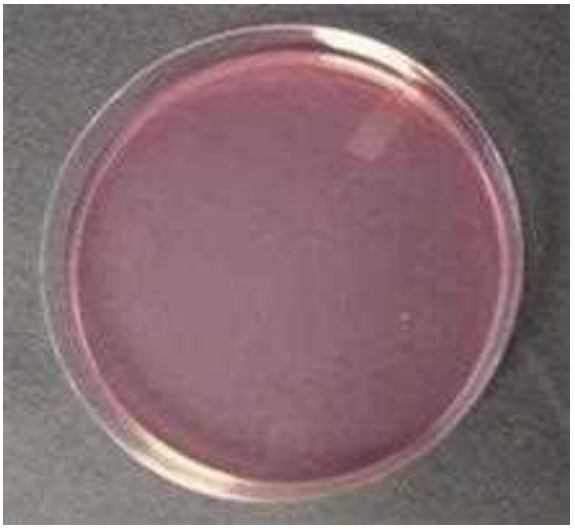
of contamination.

-Test Samples for performance, using stable, typical control cultures.

-**Shelf life**: up to sixteen weeks provided there is no change in the appearance of the medium

to suggest contamination or deteroriation

-pH of the medium 7.2-7.6 at room temperature



Nutrient agar:

Purpose: Nutrient agar is used for the cultivation of a wide variety of non-fastidious

bacteria. It was originally developed in recognition of the need for a standardized medium for use in the examination of water and waste water, dairy products and various foods. Currently it is used as a maintenance

medium for Staphylococcus aureus, Proteus mirabilis, and Escherichia coli. Tube slants are used primarily for the cultivation and maintenance of pure cultures.

Procedure for preparation:

(i) Suspend 23 g of the powder in 1 litre of purified water.

(ii) Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

(iii) Autoclave at 121˚C for 15 minutes.

(iv) Cool to 45-50C.

(v) Pour 15-20 ml of the ready media into sterile 20ml glass universal tubes.

(vi) Leave standing for thirty minutes to solidify, resting the tubes leaning at 30˚- 60˚ to

produce the slope effects in the tubes..

(vii) Perform sterility testing as described in Section 7.

(viii) Label the side of each tube with date of preparation and batch number.

(ix) Store the tubes at 2-80C sealed in plastic bags to reduce chances of contamination.

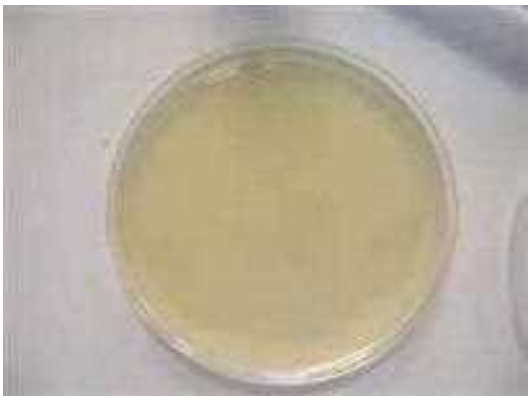
(x) Test Samples of the finished product for performance, using stable, typical control

Cultures .

-**Shelf life**: up to eighteen months provided there is no change in the appearance of the

medium to suggest contamination or deteroriation.

-pH of the medium 7.2-7.6 at room temperature.



AREAS OF RESEARCH IN ICMR:

These mediums are used for the culturing and isolation of pure colonies of Microbes which are isolated from the Animal samples.

ISOLATION OF DNA FROM BACTERIA:

Bacteria is collected from the liquid broth medium.

Materials required:

-Overnight culture of coli in LB

-TE buffer (10mM tris.Cl, 1mM EDTA, pH 8.0)

-10% SDS

-1:1 Phenol-Chloroform mixture

-Chloroform

-5N NaCl

-5M Ammoniun acetate

-Ice cold isopropanol

-70% ethanol

Protocol of DNA extraction from E.coli:

-Take 1.5 ml of bacterial broth culture (overnight culture of coli in LB) into a microfuge tube.

-Centrifuge at 800rpm for 10 minutes at 4°C and discard the supernatant.

-Suspend the pellet in 400µl TE buffer. Mix well by vortexing.

-Add 10µl of 10% SDS and mix it.

-Incubate the tube at 37°C for 1 hour in water bath.

-Shear the cell suspension 3-5 times with the help of 26G needle.

-Add 500 µlof 1:1 phenol-chloroform mixture.

-Centrifuge at 13000rpm for 2 minutes at 4°

-Transfer the supernatant into another microfuge tube.

-Add 500 µl of chloroform and centrifuge at 13000rpm for 2 minutes at 4°

-Transfer the supernatant into another microfuge tube.

-Add 25µl of 5N NaCl and centrifuge at 13000rpm for 10 minutes at 4°

-Discard the supernatant and suspend the pellet in 100µl TE buffer.

-Incubate the tube at 37°C for 30 minutes.

-Add 10 µl of 5M ammonium acetate.

-Add 250 µl of cold isopropanol and incubate at room temperature for 5 minutes.

-Centrifuge at 13000rpm for 10 minutes at 4°

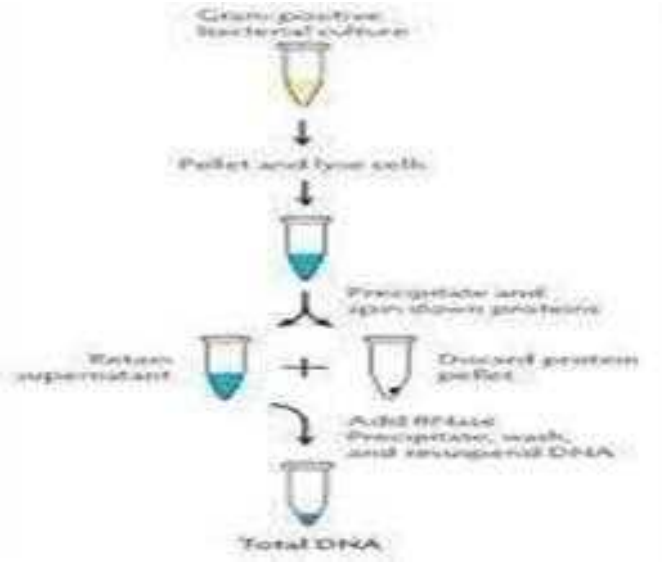
-Discard the supernatant and wash the pellet with 100 µl of 70% ethanol.

-Pour off the ethanol and invert the tube on a clean absorbent paper to drain.

-Allow the pellet to air dry for 5-10 minutes.

-Suspend the pellet in 100µl TE buffer.

-Store at -20°.



Polymerase Chain Reaction(PCR)

PCR or Polymerase Chain Reaction is a technique used in molecular biology to create several copies of a certain DNA segment. This technique was developed in 1983 by Kary Mullis, an American biochemist. PCR has made it possible to generate millions of copies of a small segment of DNA. This tool is commonly used in the molecular biology and biotechnology labs.

Principle of PCR

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA Polymerase synthesises new strands of DNA complementary to the template DNA. The DNA polymerase can add a nucleotide to the pre-existing 3'-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3' prime end of the DNA polymerase.

Components Of PCR

Components Of PCR constitutes the following:

-DNA Template– The DNA of interest from the sample.

-DNA Polymerase– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.

-Oligonucleotide Primers- These are the short stretches of single-stranded DNA complementary to the 3' ends of sense and anti-sense strands.

-Deoxyribonucleotide triphosphate– These provide energy for polymerization and are the building blocks for the synthesis of DNA. These are single units of bases.

-Buffer System– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability.

PCR Steps

The PCR involves three major cyclic reactions:

Denaturation: Denaturation occurs when the reaction mixture is heated to 94°C for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA.

The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

Annealing: The reaction temperature is lowered to 54-60°C for around 20-40 seconds. Here, the primers bind to their complementary sequences on the template DNA.

-Primers are single-strand sequences of DNA or RNA around 20 to 30 bases in length. They serve as the starting point for the synthesis of DNA.

-The two separated strands run in the opposite direction and consequently there are two primers- a forward primer and a reverse primer.

Elongation: At this step, the temperature is raised to 72-80°C. The bases are added to the 3' end of the primer by the Taq polymerase enzyme.

-This elongates the DNA in the 5' to 3' direction. The DNA polymerase adds about 1000bp/minute under optimum conditions.

-Taq Polymerase can tolerate very high temperatures. It attaches to the primer and adds DNA bases to the single strand. As a result, a double-stranded DNA molecule is obtained.

-These three steps are repeated 20-40 times in order to obtain a number of sequences of DNA of interest in a very short time period.

AGAROSE GEL ELECTROPHORESIS

Introduction:

-Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode.

-Shorter DNA fragments migrate through the gel more quickly than longer ones. Thus, you can determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known

lengths)

Equipment:

-Casting tray, -Well combs, -Voltage source, -Gel box, -UV light source, -Microwave

Reagents:

-TAE (recipe here), -Agarose, -Ethidium bromide (stock concentration of 10

mg/mL)**Procedure:**

-Pouring a Standard 1% Agarose Gel:

-Measure 1 g of agarose.

-Mix agarose powder with 100 mL 1xTAE in a microwavable flask. See TAE Recipe.

-Microwave for 1-3 min until the agarose is completely dissolved (but do not overboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up.).

-Concentration of approximately 0.2-0.5 μg/mL (usually about 2-3 μl of lab stock solution per 100 mL gel). EtBr binds to the DNA and allows you to visualize the DNAunder ultraviolet (UV) light.

-Pour the agarose into a gel tray with the well comb in place.

-Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

-Loading Samples and Running an Agarose Gel:

-Add loading buffer to each of your DNA samples.

-Once solidified, place the agarose gel into the gel box (electrophoresis unit).

-Fill gel box with 1xTAE (or TBE) until the gel is covered.

-Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel.

-A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.

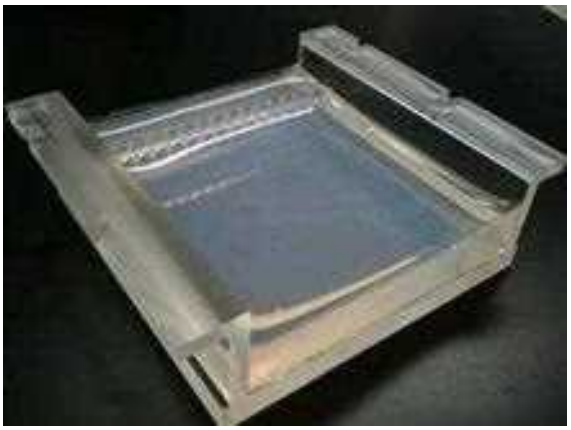
-Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box Using any device that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.

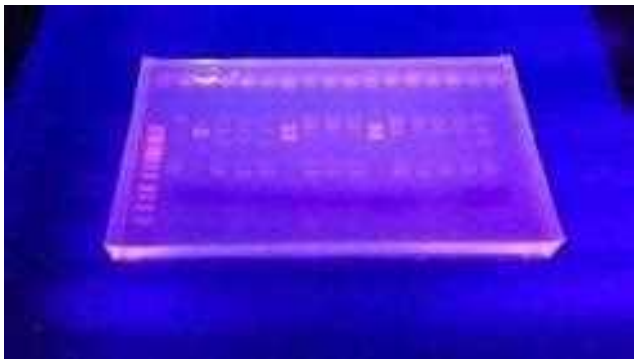
Analyzing Your Gel:

-Using the DNA ladder in the first lane as a guide (the manufacturer's instruction will tell you the size of each band), you can infer the size of the DNA in your sample lanes. For more details on doing diagnostic digests and how to interpret them please see the Diagnostic Digest page.

Purifying DNA from Your Gel:

-If you are conducting certain procedures, such as molecular cloning, you will need to purify the DNA away from the agarose gel. For instructions on how to do this, visit the Gel Purification.





AREAS OF RESEARCH USED IN ICMR:

After the extraction of the DNA from the bacterial cells the DNA is amplified by using the PCR technique. After the completion of PCR many cycles are formed , the formed DNA is run under the GEL ELECTROPHORESIS and examined under UV TRANSILLUMINATOR.

ENZYME LINKED IMMUNOSORBENT ASSAY {ELISA}

- ELISA is the basic assay technique, known as enzyme-linked immunosorbent assay (also referred to as EIA: Enzyme Immunoassay) that is carried out to detect and measure antibodies, hormones, peptides and proteins in the blood.

-Antibodies are blood proteins produced in response to a specific antigen. It helps to examine the presence of antibodies in the body, in case of certain infectious diseases.

-ELISA is a distinguished analysis compared to other antibody-assays as it yields quantitative results and separation of non-specific and specific interactions that take place through serial binding to solid surfaces, which is normally a polystyrene multiwell plate.

Principle of ELISA:

ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide useful measurement of antigen-antibody concentration.

Types Of ELISA:

-ELISA tests can be classified into three types depending upon the different methodsused for binding between antigen and antibodies, namely:

Indirect ELISA – Antigen is coated to the microtiter well

Sandwich ELISA – Antibody is coated on the microtiter

well

Competitive ELISA – Microtiter well which is antigen-coated is filled with the antigenantibody mixture.

Indirect ELISA:

-Indirect ELISA detects the presence of an antibody in a sample.

-The antigen is attached to the wells of the microtitre plate.

-A sample containing the antibodies is added to the antigen-coated wells for binding with the antigen.

-The free primary antibodies are washed away and the antigen-antibody complex is detected by adding a secondary antibody conjugated with an enzyme that can bind with the primary antibody.

-All the free secondary antibodies are washed away. A specific substrate is added which gives a coloured product.

Sandwich ELISA:

-Sandwich ELISA helps to detect the presence of antigen in a sample.

-The microtitre well is coated by the antibody.

-The sample containing the antigen is added to the well and washed to remove free antigens.

- Then an enzyme-linked secondary antibody, which binds to another epitope on the antigen is added. The well is washed to remove any free secondary antibodies.

The enzyme-specific substrate is added to the plate to form a coloured product, which can be measured.

Competitive ELISA:

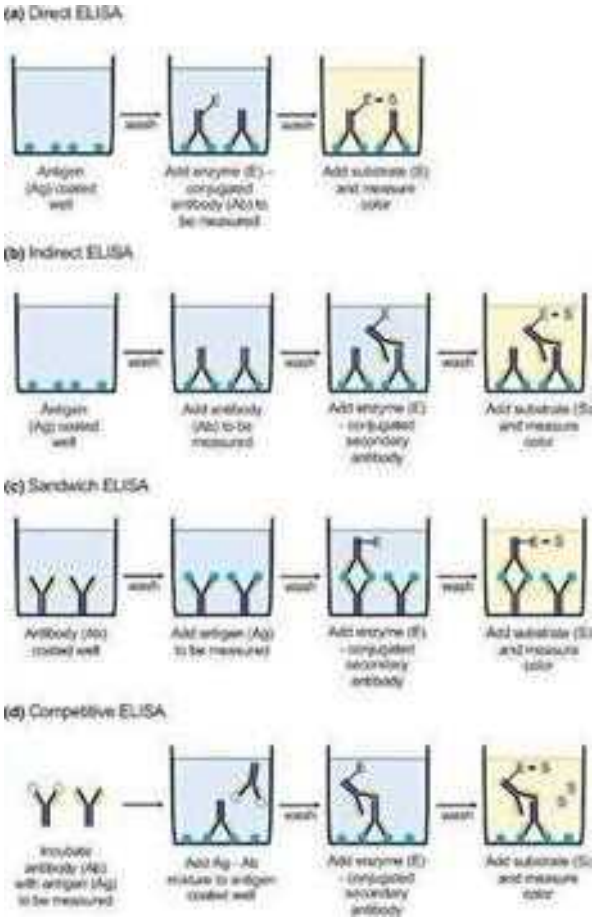
-Competitive ELISA helps to detect antigen concentration in a sample.

-The microtitre wells are coated with the antigen.

-Antibodies are incubated in a solution having the antigen.

-The solution of the antigen-antibody complex is added to the microtitre wells. The well is then washed to remove any unbound antibodies.

-More the concentration of antigen in the sample, lesser the free antibodies available to interact with the antigen, which is coated in the well.



AREAS OF RESEARCH USED IN ICMR:

Blood samples are collected from the Animals and serum is separated and examined the Anitigen and Antibody Intaration by using this ELISA technique.

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