R. J. COLLEGE of Arts, Science & Commerce (AUTONOMOUS)

(Hindi Vidya Prachar Samiti's RAMNIRANJAN JHUNJHUNWALA COLLEGE of Arts, Science & Commerce)
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Affiliated to UNIVERSITY OF MUMBAI || NAAC Re-Accredited 'A' Grade (CGPA: 3.50)

Department of Biotechnology

On Job Training Completion Report

This is to certify that Ms Divya Rao has completed On Job Training at

Ostic Pharma Pvt Ltd, SINE, IIT Bombay

Date of Commencement	Date of Completion	Total Number of Days	Total Number of Hours completed in OJT
27/12/23	6/2/24	31	62

Name of the Guide/ PI/ Incharge : Dr. Vinay Sahu

Phone Number of Guide/ PI/ Incharge : 8657104859

Email Address of the Guide/ PI/ Incharge : info.ostic@gmail.com

Ramniranjan Jhunjhunwala College NAAC Re-Accredited "A" Grade Ghatkopar, Mumbai-86

Vinay Sahu

Signature of Guide/ PI/ Incharge

OSTIC PHARMA PRIVATE LIMITED SINE, IIT BOMBAY, POWAI, MUMBAI 400076.

Gupta 22-03-24

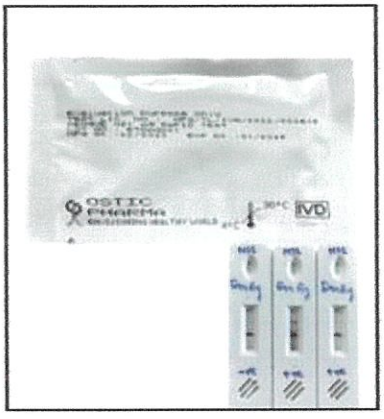
Stamp

Divya Rao
Roll no. 403

REPORT: ON-JOB TRAINING

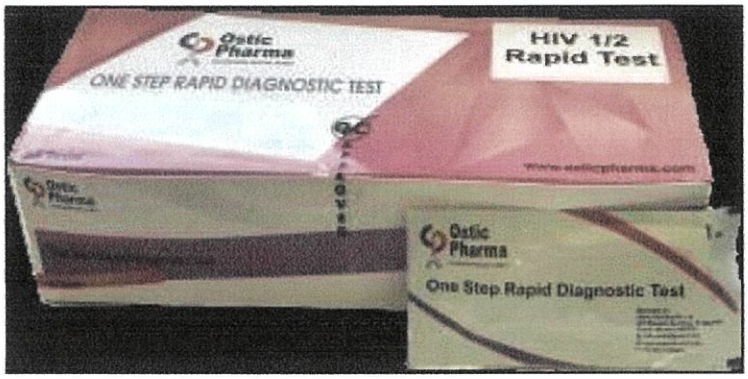
Ostic Pharma Pvt Ltd is a startup incubated with SINE (Society for Innovation & Entrepreneurship), IIT Bombay, Mumbai, Maharashtra. It is an FDA and CDSCO approved facility dedicated to promote both disruptive & incremental innovation for the betterment of society. Their goal is to develop & provide affordable technology and products that improve people's health & well-being with a special focus on the needs of vulnerable populations. They manufacture and supply various in-vitro diagnostic test kits. They also manufacture a nutraceutical product named as "Ostisterone".

During my tenure at Ostic Pharma, the first task I did was understanding the medical device rules. Medical device rules are regulations and standards governing the development, manufacturing and marketing of medical devices. These rules aim to ensure the safety, effectiveness, and quality of medical devices. Then I learnt about various diagnostic kits manufactured by the company. The Dengue NS1 Rapid Test utilizes a lateral flow chromatographic immunoassay to qualitatively detect Dengue NS1 Antigen (DEN1, 2, 3, 4) in human serum and plasma.



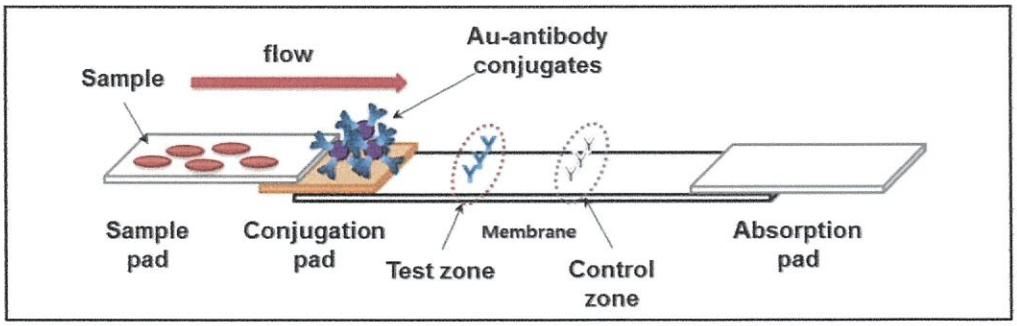
Dengue rapid test kit

The HBsAg Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum or plasma samples. The HCV Ab Rapid Test detects anti-hepatitis C virus (HCV) antibodies (IgG, IgM, IgA) in human serum or plasma, aiding in the diagnosis of HCV infection. The Malaria Pf/Pv Ag Rapid Test is a lateral flow chromatographic immunoassay that simultaneously detects and differentiates *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) antigens in human blood samples. The Syphilis Ab Rapid Test detects antibodies to *Treponema pallidum* (Tp) in human serum or plasma, aiding in the diagnosis of infection. The One Step Pregnancy Test Device is a convenient and rapid method for detecting HCG hormone in human urine, providing early pregnancy detection results. With its easy-to-use format, it offers a visual display of results within a short time frame. The HIV 1/2 Rapid Test is a single-use immunoassay that quickly identifies and distinguishes antibodies to HIV-1 and HIV-2 in human serum, plasma, or whole blood samples.



HIV rapid detection kit

The One Step COVID-19 Antigen Detection Test is an immune-chromatographic assay designed for the qualitative detection of SARS-CoV-2 antigen using nasopharyngeal swabs from individuals. The lateral flow immunoassay technology uses nitrocellulose membrane, coloured nanoparticles (or labels), and typically antibodies, to produce results. When a sample is added, the sample will flow along the test device passing through the conjugate pad into the nitrocellulose membrane and then onto the absorbent pad. Sample pad acts as the first stage of the absorption process, and in some cases contains a filter, to ensure the accurate and controlled flow of the sample. Conjugate pad stores the conjugated labels and antibodies, and will receive the sample. It aids in controlled release of re-solubilized conjugate onto the nitrocellulose membrane. Nitrocellulose membrane provides the ideal solid phase for immobilizing test and control line reagents. As the sample moves along the device the binding reagents situated on the nitrocellulose membrane will bind to the target at the test line. A coloured line will form and the density of the line will vary depending on the quantity of the target present. Absorbent pad provides uniform capillary flow through the membrane, absorbs applied sample, and prevents backflow.



Lateral flow immunoassay

I got to observe the manufacturing process of COVID-19 Antigen detection test kits. Antibodies that can accurately target COVID-19 antigens are selected, high sensitivity and specificity is ensured. Antibodies are conjugated with gold nanoparticles, to visually indicate the presence of the virus. A nitrocellulose membrane strip is prepared and coated with capture antibodies along a designated test line. Control antibodies are applied along a control line to validate the test process. Sample pad, where the patient's specimen is applied, and an absorbent pad, which helps draw the sample across the nitrocellulose strip, are carefully

prepared. These individual components are assembled onto a backing card, forming the lateral flow device structure. Then these strips are put in cassettes. I got to insert these strips onto the cassettes. The cassettes are then sealed and are integrated into the overall diagnostic kit, which includes necessary accessories like buffer solution, swabs along with clear instructions for use. I also assembled these kits. Then the kits are carefully packaged using materials that maintain the stability of the antibodies and other components, protecting them from environmental factors. Random samples from each batch undergo thorough testing to confirm the accuracy and reliability of the lateral flow immunoassay.

"SOP mastercopy" is the primary or authoritative version of a Standard Operating Procedure (SOP). The mastercopy is the original, official, or reference version of SOP, often used as a template for distribution or replication while maintaining consistency across different copies or versions. I got to read all the "SOP mastercopy" files of the company. A Device Master File (DMF) is a file that provides confidential information about a medical device or component. It's used to support pre-market submissions and may be referenced by multiple parties without disclosing its contents. I read DMF of hCG detection kit. I also made pack insert for urine test strip. Urine strip 10p is a test strip with several reagents intended for semi quantitative determination of for one or more analytes in human urine which will be launched soon. Pack insert is a sort of manual which has all the information about the kit. I also represented the company at CIIA innovation exhibition. At this exhibition, startups display their innovation projects and get exposure to industry houses, manufactures, research institutions, venture capitalists, angel investors, industry experts, etc.

Overall it was a good experience and I am thankful to Ostic Pharma Pvt Ltd for giving me the opportunity to work there.

Report by

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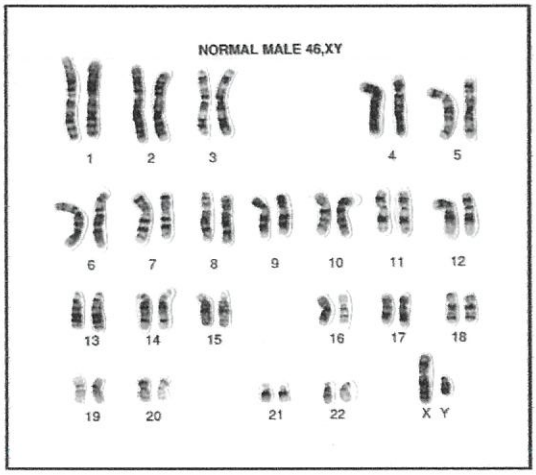
KARYOTYPING TRAINING REPORT

Karyotyping is a test to examine chromosomes in a sample of cells. This test can help identify genetic problems as the cause of a disorder or disease. The test can be performed on almost any tissue. The sample is cultured and allowed to grow in the laboratory. Cells are later taken from the new sample and stained. Using microscope, size shape and number of chromosomes in the cell sample can be analysed. The stained sample is photographed to show the arrangement of the chromosomes. This is called a karyotype.



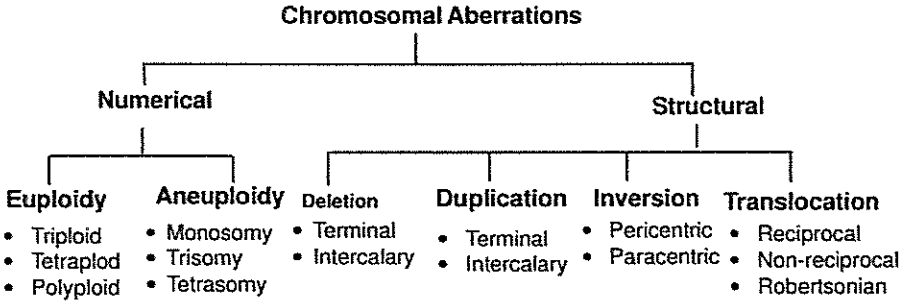
Karyotyping is used to analyze an individual's chromosomes for various purposes, including identifying genetic disorders and assessing chromosomal abnormalities. It is commonly employed in prenatal testing, cancer diagnosis, and understanding genetic conditions.

The study of chromosomes using traditional cytogenetic techniques requires cells that are actively dividing chromosomes are individually distinguishable under the light microscope, only during cell division and are best examined during metaphase. Specimens that contain spontaneously proliferating cells include: bone marrow, lymph nodes, solid tumors, tissue biopsies, amniotic fluids, chorionic villi. Peripheral blood samples should be collected in sterile syringes or vacuum tubes containing preservative-free sodium heparin for best results, blood samples should be set up within 24 hours of collection and temperature extremes must be avoided if samples are transported or stored. Specimens should be kept at room temperature or refrigerated above 4°C until they can be processed a repeat sample should be requested if these requirements are not met (e.g., the sample is received clotted, on ice, more than 24 hours old). Bone marrow aspirates should be collected in sterile syringes or vacuum tubes containing preservative-free sodium heparin and transported at room temperature. Bone marrow specimens should be processed immediately upon receipt to avoid cell death. Amniotic fluid specimen collection can be performed from as early as 10 weeks of gestation until term .15 to 30 millilitre of amniotic fluid is obtained under sterile conditions and

collected in a sterile container approved for cell culture. Samples are transported at room temperature. Temperature extremes and long transport times should be avoided.

Growth media such as AmnioMAX™, chang medium or amniochrome is used for amniocytes, giant cell tumor-conditioned medium for malignancies, PANDIS for breast tumors while others are appropriate for a broad spectrum of cell types (e.g., RPMI 1640, MEM). All culture media are balanced salt solutions with a variety of additives including salts, glucose, and a buffering system to maintain the proper pH. Phenol red is often used as a pH indicator in many media. If the medium becomes too acidic, it will turn yellow, while medium that is too basic becomes pink or purple. L-Glutamine is an amino acid essential for cell growth. L-Glutamine is unstable and breaks down on storage to D-glutamine, a form that cannot be used by cells. L -Glutamine must therefore be stored frozen to retain its stability, and it is optimal to add it to the culture medium just prior to use. Serum is essential for good cell growth. Fetal bovine serum (FBS) is preferred; culture medium is generally supplemented with 10–30% FBS. Microbial inhibitors are added to culture media to retard the growth of microorganisms. Penicillin/streptomycin, kanamycin, and gentamicin are bacterial inhibitors commonly used in tissue culture. Some cells, particularly mature lymphocytes, do not spontaneously undergo cell division and must be stimulated to divide by the addition of an appropriate mitogen to the cell culture. Phytohemagglutinin (PHA) is an extract of red kidney beans that stimulates division primarily of T-lymphocytes.

Chromosomal aberrations are broadly classified as numerical or structural aberrations.



Numerical aberrations are those that cause a change (addition or deletion) in the number of chromosomes. They can be further classified as euploidy changes or aneuploidy changes. Euploidy is the condition when an organism gains or losses one or more complete set of chromosomes, thus causing change in the ploidy number. For example, triploid (3n), tetraploid (4n) etc. Aneuploidy is the condition when an organism gains or losses one or more chromosomes and not the entire set. For example, trisomy (2n + 1), monosomy (2n – 1). In humans, euploidy conditions do not exist because the extent of abnormality is too large to sustain life. Aneuploidy conditions, however, are more common and are manifested in disorders such as Down syndrome, Klinefelter syndrome and Turner syndrome. Structural aberrations are those that involve a change in the chromosome structure. These include

deletions, duplications and rearrangements (inversions and translocations). Structural changes occur when chromosomes break and later rejoin in combinations that are different from the original. Trisomy is the condition where there is an additional copy of one chromosome. It is represented as 2n+1. Individuals, who are trisomics, thus show three copies of the chromosome rather than the normal two. They cause conditions such as Down syndrome (trisomy 21), Edward syndrome (trisomy 18), and Patau syndrome (trisomy 13). Changes in the sex chromosome constitution causes conditions such as Turner syndrome (monosomy X – XO) and Klinefelter syndrome (XXY). Numerical aberrations needn't necessarily be present in all of the affected individuals. Cases of milder symptoms have been shown to be due to mosaicism whereby only a subset of the individual's cells contains the aberration. The presence of normal cells in the individuals lessens the severity of the symptoms. The variability of the symptoms also depends on which organ's cells contain the aberration. If the aberrant chromosomal genes are not normally expressed in the organ containing cells with the aberration, no symptoms will develop. Chimerism is similar to mosaicism differing only in the origin of the different cell lines being from different zygotes. Structural aberrations include deletions, duplications, translocations and inversions. They also include isochromosome and ring chromosome that are the cause for a small percentage of syndromes like Turner syndrome. Among the structural aberrations, it is only the deletions and duplications (unbalanced changes) that majorly cause abnormal phenotypes such as Cri-du-chat syndrome and Charcot-Marie-Tooth disorder. Balanced rearrangements (translocations and inversions) by themselves do not cause abnormal phenotype because there is no actual loss or gain of genes – only their rearrangement. They, however, cause deletions and duplications in the next generation.

Banding technique is a technique for the identification of chromosomes and its structural abnormalities in the chromosome complement. Chromosome identification depends on their morphological characteristics such as relative length, arm ratio, presence and absence of secondary constrictions on the chromosome arms. On banded chromosome, darkly stained or brightly fluorescent transverse bands (positive bands) alternate with the lightly stained or less fluorescent (negative bands). The bands are consistent, reproducible and are specific for each species and each pair of homologous chromosomes. The three banding techniques in which bands are distributed along the length of the whole chromosome are giemsa banding (G-banding), quinacrine banding (Q-banding) and reverse binding (R-banding).

For cell culturing, I collected the blood sample in sterile tubes. Then RPMI 1640 culture medium, FBS, PHA which induces mitosis was added. Then the tubes were incubated for 70 hours at 37^0 C. After incubation colchicine was added which arrests cells in metaphase, then again tube was incubated for 1 hour. Then the tube was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded and KCl was added to the pellet. Osmosis causes inflammation of cell, dispersing chromosomes. Then the tube was incubated at 37^0C for 20 minutes. For fixation, the tube was again centrifuged at 1000 rpm for 10 minutes. The

supernatant was discarded and fixative was added (1:3 glacial acetic acid: methanol) with simultaneous mixing of the pellet and the tube was refrigerated overnight. Next day, the tube was removed from the refrigerator, centrifuged and 2-3 washes were given with fixative till the pellet became white. At the end supernatant was discarded. The pellet was mixed thoroughly and dropped on the cold slide from a height and kept on the hot plate to heat dry. The slide was then stained with Giemsa stain (G-banding) and chromosomes were viewed under the microscope.

This training helped me to improve my lab skills and I gained knowledge about chromosome abnormalities and basics of animal tissue culture. I am thankful to Dr Sucheta Golwalkar ma'am and Dr Posam ma'am for the opportunity.

Report by
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