BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,

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Programme: M.Sc., Biomedical Science

Course Code: 18BMS59C17

Course Title: Immune & Molecular Diagnostics

Unit-V

Molecular Diagnostics

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Unit V:

Molecular Diagnostics- Diagnosis of Mycobacterim tuberculosis, HCV & HIV; Conventional vs Molecular Diagnostics- Merits & Demerits. Biological warfare: Bacillus anthracis, H5N1, SARS- CoV, Chikunguniya- pathogenesis- diagnostic methods- Molecular diagnosis of single gene disorder- sickle cell anemia- Molecular HLA typing- Sequence Specific PCR (SSP), Sequence Specific oligonucleotide probe (SSOP), Sequence Based Typing (SBT)- Advantages of molecular HLA typing over serological methods- HLA typing and clinical significance- comment on Sensitivity and Specificity of clinical laboratory techniques- Quality assessment Programs (external & internal assessment Programs)

PRESENTATION: 3

ADVANTAGES OF MOLECULAR HLA TYPING AND CLINICAL SIGNIFICANCE

HLA TYPING

- **HLA typing** (Human Leukocyte Antigen typing) is a laboratory procedure used to identify the specific variations of HLA genes that a person possesses.
- HLA genes encode proteins on the surface of cells that play a crucial role in the immune system's ability to distinguish between the body's own cells and foreign invaders, such as viruses or bacteria.

ADVANTAGES OF HLA TYPING

Higher Resolution and Accuracy:

- Molecular HLA typing provides a much more precise identification of HLA alleles at the DNA level.
- This high resolution helps in identifying specific alleles and distinguishing between closely related HLA types, which is not possible with serological methods.

Improved Matching for Transplantation:

- Accurate HLA typing is crucial for matching organ and bone marrow donors with recipients. Molecular typing reduces the risk of graft rejection and increases the chances of transplant success by ensuring a better match.

Detection of Rare Alleles:

- Molecular techniques can identify rare and new alleles that might not be detectable by serological methods, leading to a more comprehensive understanding of the HLA gene pool.

Standardization and Reproducibility:

- Molecular typing methods are standardized, making them more reproducible across different laboratories.
- This consistency is vital for applications in clinical settings and research.

Applicability to Different Sample Types:

- Molecular typing can be performed on a variety of sample types, including blood, buccal swabs, and even small amounts of DNA.
- This flexibility makes it more convenient and less invasive for patients.

Automation and High Throughput:

- Molecular HLA typing can be automated and scaled for high-throughput processing, which is particularly useful in large-scale studies or when typing large numbers of samples.

Decreased Cross-Reactivity Issues:

- Serological methods can suffer from cross-reactivity, where antibodies react with multiple HLA antigens, leading to ambiguous results. Molecular typing eliminates these issues by directly analyzing the DNA sequence.

CLINICAL SIGNIFICANCE

Organ and Stem Cell Transplantation:

- **Graft Compatibility**: HLA typing is critical for matching organ and stem cell donors with recipients. A close HLA match between donor and recipient reduces the risk of graft rejection and improves the overall success of the transplant.
- **Graft-Versus-Host Disease (GVHD)**: In bone marrow or stem cell transplantation, a close HLA match helps prevent GVHD, a potentially life-threatening condition where the donated cells attack the recipient's body.

Disease Association:

➢ Autoimmune Diseases:

Certain HLA types are associated with an increased risk of developing autoimmune diseases.

For example:

- **HLA-B27** is strongly associated with ankylosing spondylitis and other spondyloarthropathies.
- **HLA-DR3 and HLA-DR4** are associated with a higher risk of type 1 diabetes.
- **HLA-DQ2 and HLA-DQ8** are linked to celiac disease.

Paternity and Forensic Testing:

- HLA typing can be used in paternity testing to confirm biological relationships.
- It is also used in forensic medicine to help identify individuals or establish kinship in cases of disputed identity.

Transfusion Medicine:

- **Platelet Transfusions**: HLA typing is important in patients who require frequent platelet transfusions.
- These patients may develop antibodies against foreign HLA antigens (alloimmunization), making it difficult to find compatible donors. HLA-matched platelets are often required in such cases.

Personalized Medicine:

- HLA typing plays a role in personalized medicine, where treatment is tailored to the genetic makeup of the individual.
- For example, selecting the most appropriate immunosuppressive therapy post-transplantation may depend on the patient's HLA type.

HLA Typing in Infectious Diseases:

- Certain HLA types influence the susceptibility to infectious diseases and the course of the disease.
- For example, individuals with certain HLA types may have a different response to infections like HIV or hepatitis.

Population Genetics and Anthropology:

- HLA typing is also used in population genetics to study the distribution of HLA alleles across different populations.
- This information is valuable in understanding population history, migrations, and evolutionary pressures.

Vaccine Development:

- Understanding HLA-associated immune responses helps in the design of vaccines that are more effective across different populations by accounting for the variability in HLA types.

SENSITIVITY AND SPECIFICITY OF CLINICAL LABORATORY TECHNIQUES

- **Sensitivity** and **specificity** are critical parameters used to evaluate the performance of clinical laboratory techniques.
- These metrics help in determining the reliability of a test in identifying true positives and true negatives, respectively.

1. Sensitivity

Definition:

- Sensitivity is the ability of a test to correctly identify individuals who have a specific disease or condition (true positives). It measures the proportion of actual positives that are correctly identified by the test.

Formula:

$$\text{Sensitivity} = \frac{\text{True Positives (TP)}}{\text{True Positives (TP)} + \text{False Negatives (FN)}}$$

Clinical Implication:

- A highly sensitive test minimizes the number of false negatives, which is crucial for diseases where missing a diagnosis could lead to severe consequences (e.g., infectious diseases like HIV, cancer screening).

Example:

- If a test for detecting tuberculosis has a sensitivity of 95%, it means that 95% of patients with tuberculosis will be correctly identified by the test.

Application:

- **Screening Tests:** High sensitivity is crucial in screening tests to ensure that most of the affected individuals are identified and can undergo further diagnostic evaluation.

Challenges:

- High sensitivity may sometimes lead to a higher number of false positives, which can necessitate further confirmatory testing.

2. Specificity

Definition:

- Specificity is the ability of a test to correctly identify individuals who do not have the disease (true negatives). It measures the proportion of actual negatives that are correctly identified by the test.

Formula:

$$\text{Specificity} = \frac{\text{True Negatives (TN)}}{\text{True Negatives (TN)} + \text{False Positives (FP)}}$$

Clinical Implication:

- A highly specific test minimizes the number of false positives, which is important to avoid unnecessary anxiety, further testing, and treatment for patients.

Example:

If a test for diagnosing rheumatoid arthritis has a specificity of 90%, it means that 90% of individuals without the disease will be correctly identified as negative.

3. Balancing Sensitivity and Specificity

Trade-Offs:

- In practice, there is often a trade-off between sensitivity and specificity. Improving sensitivity can decrease specificity and vice versa.

Receiver Operating Characteristic (ROC) Curve:

This graphical plot is used to determine the optimal balance between sensitivity and specificity for a particular test. The area under the ROC curve (AUC) reflects the overall diagnostic ability of the test.

Choosing the Right Test:

- **High Sensitivity Tests:**

Preferred in early stages of disease detection or in diseases where missing a diagnosis is critical (e.g., cancer screening, infectious diseases).

- **High Specificity Tests:**

Preferred when confirmatory diagnosis is needed to prevent false positives and unnecessary interventions (e.g., confirmatory tests for HIV or cancer).

4. Examples of Clinical Laboratory Techniques

1. Enzyme-Linked Immunosorbent Assay (ELISA):

- **Sensitivity:** High, used for detecting antibodies or antigens in infectious diseases.
- **Specificity:** Can be high but requires confirmation due to potential for cross-reactivity.

2. Polymerase Chain Reaction (PCR):

- **Sensitivity:** Extremely high, can detect minute amounts of genetic material.
- **Specificity:** High when primers are specific to the target sequence.

3. Rapid Diagnostic Tests (RDTs):

- **Sensitivity:** Varies, generally lower than PCR but higher than traditional methods.
- **Specificity:** Varies, potential for false positives, hence used as screening tools.

4. Imaging Techniques (e.g., MRI, CT Scans):

- **Sensitivity:** Depends on the condition; high in detecting structural abnormalities.
- **Specificity:** Can vary; may require corroboration with clinical findings or other tests.

5. Clinical Impact

1. Reducing Diagnostic Errors:

- Tests with appropriate sensitivity and specificity reduce the risk of diagnostic errors, leading to better patient outcomes.

2. Cost-Effectiveness:

- Properly balanced tests reduce the need for repeated testing and unnecessary treatments, optimizing healthcare resources.

3. Personalized Medicine:

- High specificity tests contribute to personalized medicine by ensuring that treatments are accurately targeted to those who will benefit.

QUALITY ASSESSMENT PROGRAMS

Quality Assessment Program divided into two:

- **External Assessment Program**
- **Internal Assessment Program**

External Assessment Program

- External assessment involves the **external provider evaluates the lab's results** and **compares them** to results from other labs.
- This helps verify that the lab meets **quality standards**.

Components

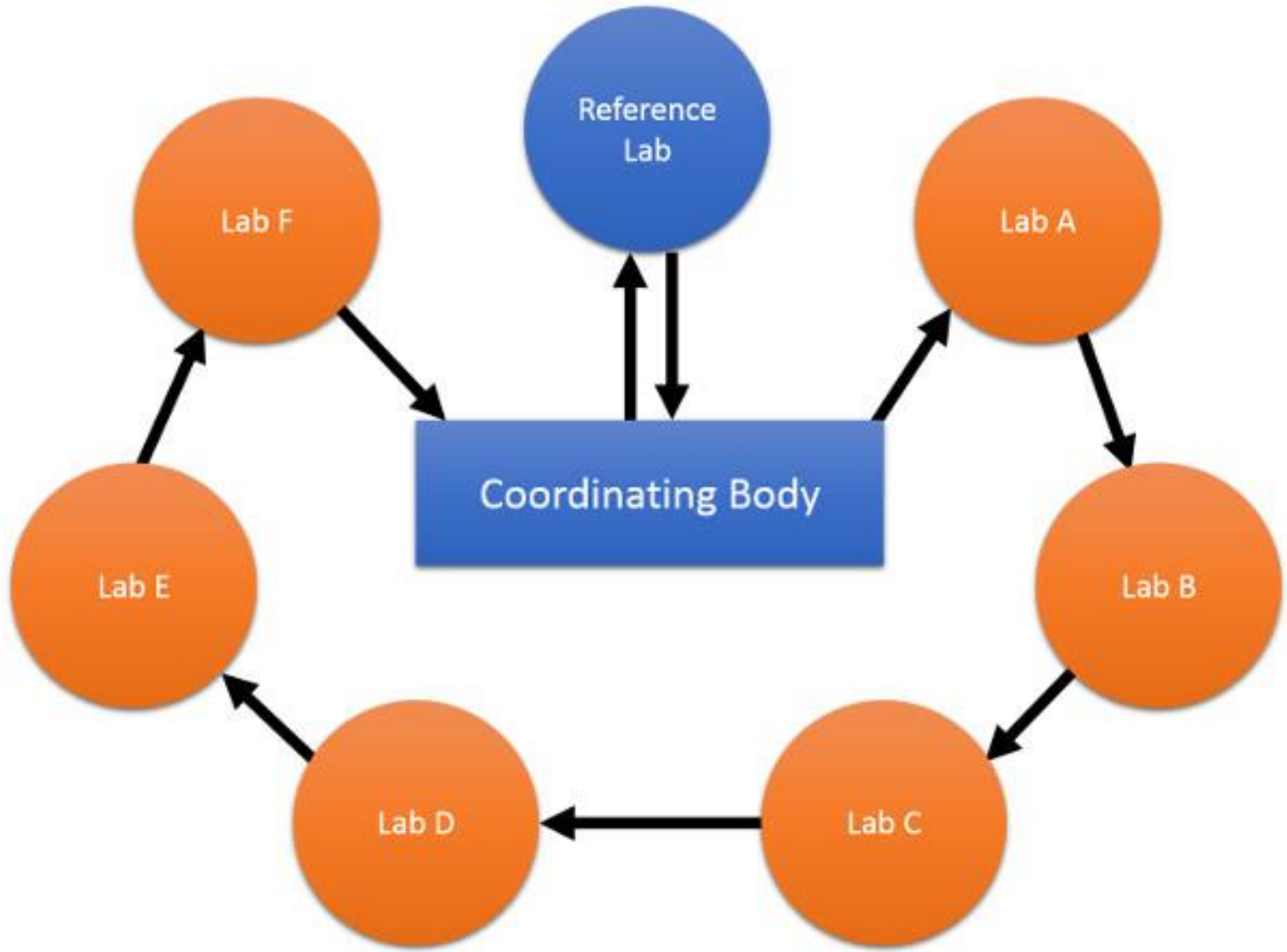
Proficiency Testing (PT):

- **Blind Samples:**

Testing unknown samples provided by an external provider to validate the accuracy of the laboratory’s methods.

Inter-laboratory Comparisons:

- Participating in external proficiency testing programs where the **same samples** are sent to different labs **to ensure consistency in results.**


Reference Lab
Lab F
Lab A
Coordinating Body
Lab E
Lab B
Lab D
Lab C

Proficiency testing results are evaluated using two methods described in ISO/IEC 17043

Individual performance indicators include:

- Your measurement data at each nominal set point
- Reference values and uncertainties of test artifact(s)
- Participant's E_n values, S/U (satisfactory/unsatisfactory) ratings, IWO (in/with[illegible]out of range) ratings and [illegible]score

Normalized Error

Z-score

Measurement Description	Reported Value / *Reference Value*	Reported Uncertainty / *Reference Uncertainty*	E_n	S/U	IWO	Z Score
2) 10.0000 MPa	10.0000 / *10.0001*	0.0010 / *0.0016*	-0.03	S	I	-0.04
3) 20.0000 MPa	20.0010 / *20.0013*	0.0020 / *0.0030*	-0.07	S	I	-0.09
4) 30.0000 MPa	30.0005 / *30.0021*	0.0030 / *0.0025*	-0.42	S	I	-0.39
5) 40.0000 MPa	39.9991 / *40.0006*	0.0040 / *0.0026*	-0.31	S	I	-0.26

External Audits:

- An external body (e.g., ISO) audits the lab's processes and protocols to ensure compliance with international standards.

PRINCIPAL INVESTIGATOR:	AUDITOR:	DATE:
DEPARTMENT:	BUILDING & ROOM(S):	
LAB REP (IF NOT PI):	PHONE:	EMAIL:

ITEM	DEFICIENCY	COMMENTS
Biosafety Resources and Documentation		http://www.grinnell.edu/academic/research-ethics/ibc
Biological research (e.g. rDNA, BSL-2 agent) is approved by IBC		
Biosafety registration is current		
Biosafety Manual is readily available (BMBL, 5th ed, 2009)		
All personnel have completed annual biosafety training		
Standard Microbiological Practices		http://www.cdc.gov/od/ohs/biosfty/biosfty.htm
Lab supervisor controls access to the laboratory		
Persons wash their hands after working with samples and before leaving the lab		
Eating, drinking, and storing food for consumption are not permitted in lab areas		
Mouth pipetting is prohibited; mechanical pipetting devices are always used		
Needles are never bent, broken, recapped or reused before disposal		
Used needles, syringes, and other sharps placed in a puncture-resistant container		
Plasticware is substituted for glass whenever possible		
All procedures are performed to minimize the creation of splashes and/or aerosols		
Work surfaces are decontaminated after completion of work or after any spill		
Biological waste (e.g. cultures, stocks) are properly decontaminated before disposal		
Samples are placed in durable, leak proof container during storage and transport		
Biological waste is stored in a durable leak proof container lined with a biowaste bag		

Laboratory Facilities		http://www.cdc.gov/od/ohs/biosfty/biosfty.htm
Laboratory has a sink for hand washing; soap and paper towels are available		
Eyewash station is readily available		
Lab designed so that it can be easily cleaned (i.e. no carpet, cloth furniture, etc.)		
Bench tops are impervious to water and resistant to heat and other chemicals		
Lab windows that open to the exterior are fitted with screens		
Housekeeping is appropriate and lab is maintained in a clean/sanitary condition		
BSL-2 Laboratory (Special Practices & Facility Requirements)		http://www.cdc.gov/od/ohs/biosfty/biosfty.htm
Biohazard signage is posted at the lab entrance when infectious agents are present		
Site specific training has been documented (Infectious agents personnel training)		
Lab equipment is routinely decontaminated, including after spills or splashes		
Biological Spill Procedures are available and personnel are familiar with procedures		
All animals and plants not associated with work being performed are prohibited		
All procedures that may generate aerosols are conducted in containment (e.g. BSC)		
Bloodborne Pathogens (i.e. human blood, body fluids, cell lines, unfixed tissues) Note: only applies to labs utilizing blood or other potentially infectious materials		http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=21010
Exposure Control Plan is accessible		
All personnel have completed annual BBP training		
All personnel have been offered Hepatitis B vaccination or signed declination form		
Personnel are familiar with post-exposure evaluation and follow-up		
Engineering and work practice controls are used to reduce the risk of exposure		
Shipping Biological Materials Note: only applies if/when regulated materials are to be shipped		http://www.grinnell.edu/academic/research-ethics/ibc
PI has completed biological materials shipping training in the past two years		
Guidelines for shipping regulated biological materials are available in the lab		
Comments:		**Biological materials used/stored:**
All deficiencies designated (M) must be corrected by:	Total deficiencies: ☐ **Pass** ≤ 4 ☐ **Fail** > 4	

Accreditation and Certification:

- **ISO Accreditation:** Labs can be accredited by organizations such as the International Organization for Standardization (ISO). Regular external audits ensure compliance with these standards.

- **GLP (Good Laboratory Practice):** For labs involved in preclinical research, adherence to GLP guidelines is essential. External audits ensure that the lab's practices meet regulatory standards.

Internal Assessment Program

- IAP focuses on the continuous monitoring and improvement of a laboratory's internal processes and test results.
- Self-evaluation process designed to monitor, evaluate, and improve an organization’s processes and outcomes.
- It ensures that daily operations meet high standards of accuracy and reliability.

Protocol Adherence

Standard Operating Procedures (SOPs)- Every procedure, from sample collection to data analysis, must follow detailed SOPs to minimize variability and errors. Regular internal audits ensure that SOPs are consistently followed.

Documentation- Accurate and detailed records of experiments, including any deviations from the protocol, are essential for reproducibility and traceability.

Equipment Calibration and Maintenance

Routine Calibration:

Instruments such as flow cytometers, ELISA plate readers, and PCR machines must be regularly calibrated to ensure accurate results. Internal assessments track maintenance schedules and performance logs.

Check and recalibrate pipettes, pH meters to maintain measurement accuracy.

Quality Control Samples:

Running control samples alongside patient or experimental samples helps detect any issues with the equipment or reagents.

Reagent and Sample Quality

- Different batches of reagents (e.g., antibodies, cytokines), so internal tests should verify consistency between lots before use in experiments.
- Run side-by-side experiments using both the old and new batches of reagents under the same conditions. Measure key performance indicators like binding **affinity for antibodies** or biological activity for cytokines.
- Regular checks on the storage conditions (e.g., temperature monitoring) of biological samples (serum, plasma, cells) to prevent degradation.

Data Validation and Analysis

Data Integrity: Ensuring that data is accurately recorded and analyzed without any manipulation. Use of validation methods such as replicate experiments or internal controls.

Statistical Analysis: Applying appropriate statistical methods to interpret data correctly. Regular internal reviews can catch errors in analysis or interpretation.

Key difference

External Quality Assessment (IQA)	Internal Quality Assessment (IQA)
External validation through interlaboratory comparisons	Internal monitoring and quality control of daily operations.
Periodic assessments (e.g., quarterly, annually).	Continuous, daily monitoring.
Benchmarks performance against external standards and other laboratories.	Ensures consistency and accuracy of internal processes and results.
Often required for regulatory compliance and accreditation.	Essential for ongoing quality management and internal process improvement.

Conclusion:

- Both EQA and IQA are crucial for maintaining high standards in clinical laboratories. EQA provides external validation and benchmarks against industry standards, while IQA ensures continuous internal quality control and improvement.
- Together, they help laboratories deliver accurate and reliable test results, ultimately improving patient care and outcomes.

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