SERUM INSTITUTE OF INDIA

Internship report – July 2022

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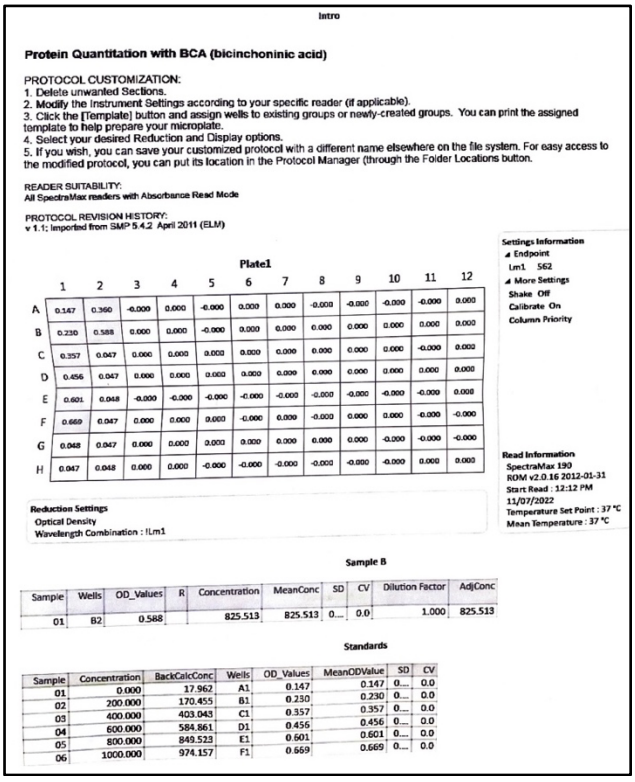
Under the guidance of Dr. Shruti Polishwalla and Dr. Umesh Shaligram

1. Recombinant lab

Mentors: Mr. Akshay Shedge, Dr. Santosh Narwade, and Mr. Suraj

Visited the recombinant lab, learned about all the equipment present, and even conducted some basic experiments.

- Dilution and concentrations
- Pipetting
- Protein quantification: to understand the total protein content in a sample via fluorescently labelled proteins (UV-spectrophotometer) or via bicinchoninic acid assay (BCA) [figure 1]
- Ultracentrifugation and analytical ultracentrifugation
- Cell lysis through osmotic shock: cells are subjected to high osmotic pressure followed by sudden dilution, causing water to rapidly enter cells and rupture the membrane to release cytoplasmic components
- Cell viability: refers to the number of live, healthy cells in a sample [figure 2]
- Isoelectric focusing
- HPLC: High performance liquid chromatography: process of separating components in a liquid mixture. A liquid sample is injected into a stream of solvent (mobile phase) flowing through a column packed with a separation medium (stationary phase) [figure 3]
- Size exclusion chromatography
- qRT – PCR: Real-time quantitative reverse transcription polymerase chain reaction [figure 4]



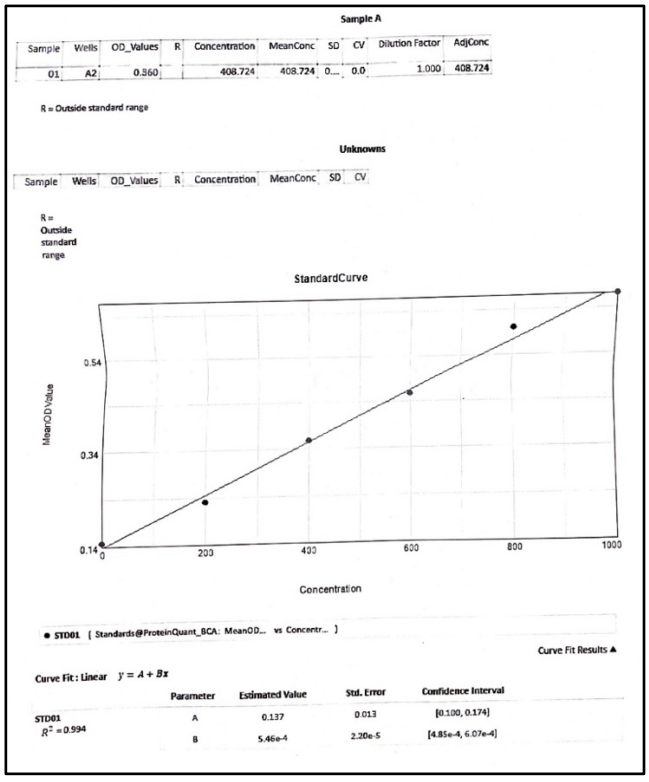
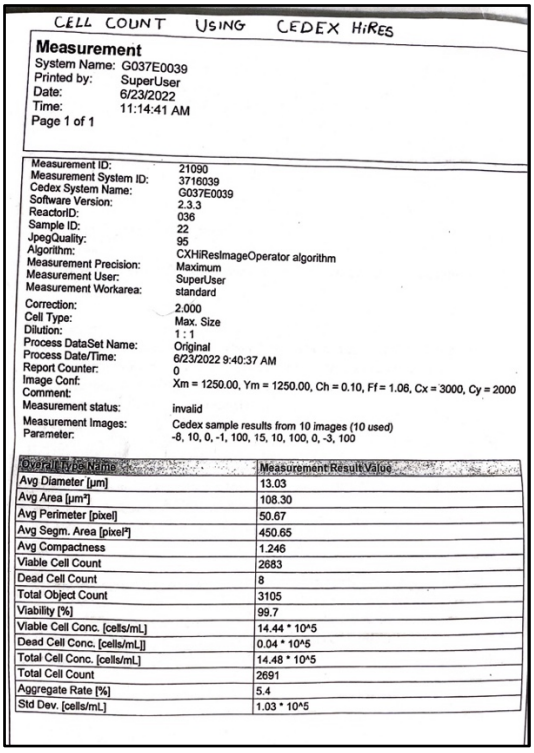


Figure 1. Protein quantification via BCA



CELL COUNT USING CEDEX HiRES

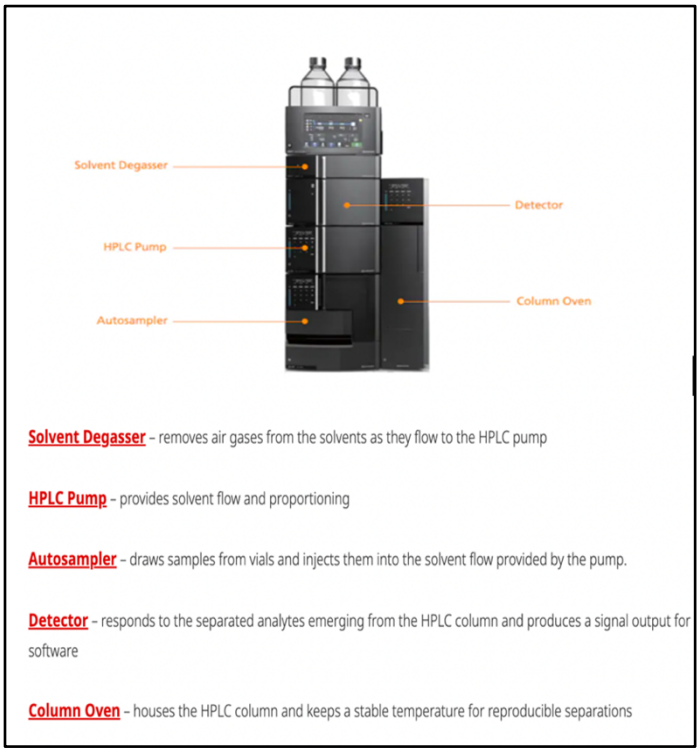
Measurement

System Name: G037E0039
Printed by: SuperUser
Date: 6/23/2022
Time: 11:14:41 AM
Page 1 of 1

Measurement ID:	21090
Measurement System ID:	3716039
Cedex System Name:	G037E0039
Software Version:	2.3.3
ReactorID:	036
Sample ID:	22
JpegQuality:	95
Algorithm:	CXHiResImageOperator algorithm
Measurement Precision:	Maximum
Measurement User:	SuperUser
Measurement Workarea:	standard
Correction:	2.000
Cell Type:	Max. Size
Dilution:	1 : 1
Process DataSet Name:	Original
Process Date/Time:	6/23/2022 9:40:37 AM
Report Counter:	0
Image Conf:	Xm = 1250.00, Ym = 1250.00, Ch = 0.10, Ff = 1.06, Cx = 3000, Cy = 2000
Comment:	
Measurement status:	invalid
Measurement Images:	Cedex sample results from 10 images (10 used)
Parameter:	-8, 10, 0, -1, 100, 15, 10, 100, 0, -3, 100

Overall Types Name	Measurement Result Value
Avg Diameter [µm]	13.03
Avg Area [µm²]	108.30
Avg Perimeter [pixel]	50.67
Avg Segm. Area [pixel²]	450.65
Avg Compactness	1.246
Viable Cell Count	2683
Dead Cell Count	8
Total Object Count	3105
Viability [%]	99.7
Viable Cell Conc. [cells/mL]	14.44 * 10^5
Dead Cell Conc. [cells/mL]]	0.04 * 10^5
Total Cell Conc. [cells/mL]	14.48 * 10^5
Total Cell Count	2691
Aggregate Rate [%]	5.4
Std Dev. [cells/mL]	1.03 * 10^5

Figure 2. Cell count via CEDEX HiRES



Solvent Degasser – removes air gases from the solvents as they flow to the HPLC pump

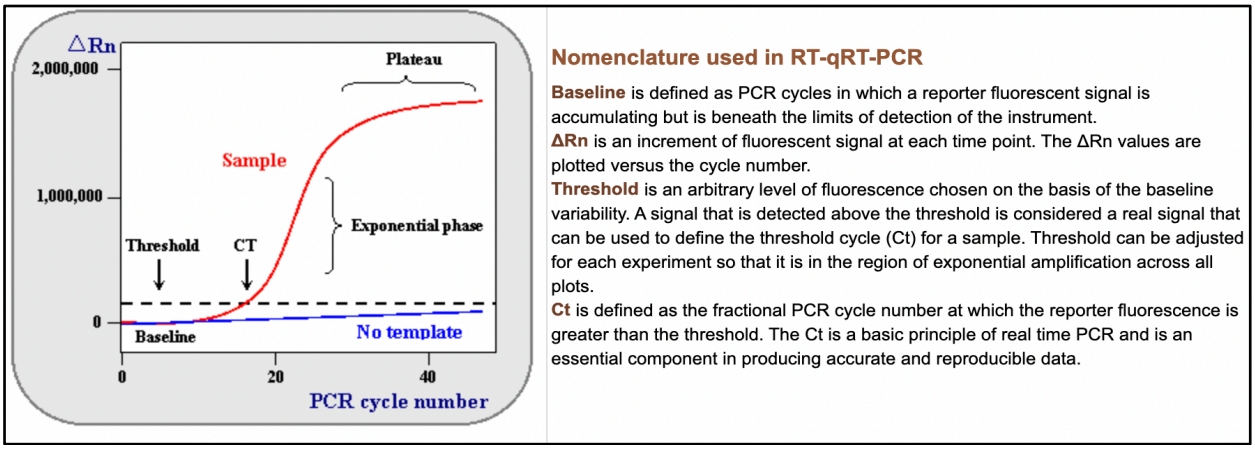
HPLC Pump – provides solvent flow and proportioning

Autosampler – draws samples from vials and injects them into the solvent flow provided by the pump.

Detector – responds to the separated analytes emerging from the HPLC column and produces a signal output for software

Column Oven – houses the HPLC column and keeps a stable temperature for reproducible separations

Figure 3. Basic HPLC system



Nomenclature used in RT-qRT-PCR

Baseline is defined as PCR cycles in which a reporter fluorescent signal is accumulating but is beneath the limits of detection of the instrument.
ΔRn is an increment of fluorescent signal at each time point. The ΔRn values are plotted versus the cycle number.
Threshold is an arbitrary level of fluorescence chosen on the basis of the baseline variability. A signal that is detected above the threshold is considered a real signal that can be used to define the threshold cycle (Ct) for a sample. Threshold can be adjusted for each experiment so that it is in the region of exponential amplification across all plots.
Ct is defined as the fractional PCR cycle number at which the reporter fluorescence is greater than the threshold. The Ct is a basic principle of real time PCR and is an essential component in producing accurate and reproducible data.

Figure 4. Model of real-time quantitative PCR plot

Learned about the process and science behind developing the Covishield vaccine.

- The ChAdOx1-S recombinant vaccine is a replication-deficient chimpanzee adenoviral vector vaccine against coronavirus disease 2019 (COVID-19). The vaccine expresses the SARS-CoV-2 spike glycoprotein gene, which instructs the host cells to produce the protein of the S-antigen unique to SARS-CoV-2, allowing the body to generate an immune response and to retain that information in memory immune cells.

2. Clinical Trials

Mentors: Dr. Dhananjay Kapse and Dr. Jyoti

- A clinical trial is a research study conducted with the goal of understanding new therapies, vaccines diagnostic procedures, or new ways of using known treatments. Clinical trials are used to determine whether new drugs, diagnostics or treatments are both safe and effective. Participants are entitled to

a clinical trial that adheres to all legal and ethical standards. They have a right to a clear, transparent Informed Consent process before they agree to join the trial and can withdraw at any time.

- Pharmacovigilance: the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine/vaccine related problem.

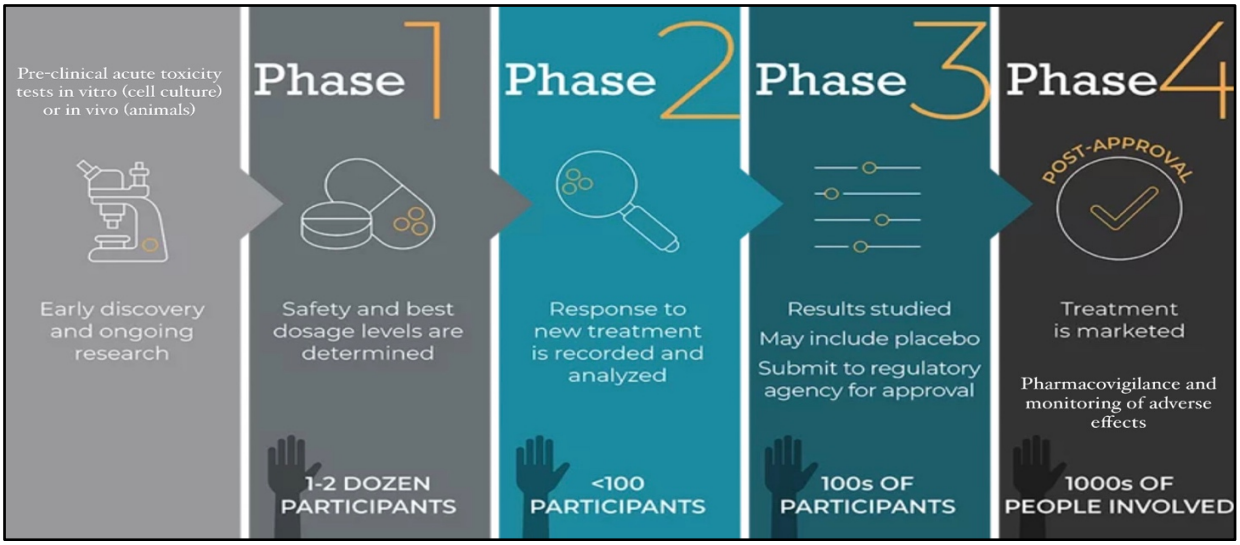


Figure 5. Phases of clinical trials

Read the International Council for Harmonization (ICH) guidelines for Good Clinical Practice (GCP) and learned about trial design, protocols, informed consent, and study reports.

- GCP is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, and that the clinical trial data are credible.

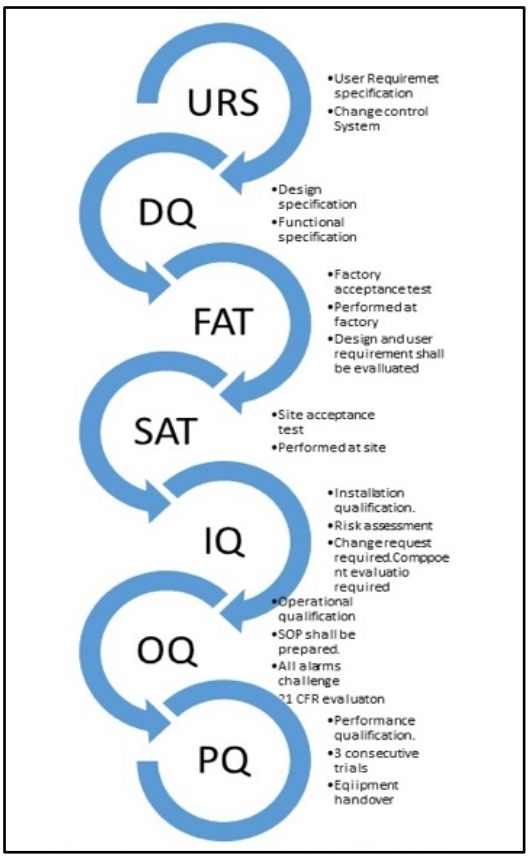
ICH: GUIDELINE FOR GOOD CLINICAL PRACTICE

3. Quality assurance

Mentor: Mr. Rahul Deshpande

Discussed about the correlation between quality and cost, immune responses, patented and generic medicines, and the multiple costs involved in a business (R&D, labour, manufacturing)

- Quality is a measure of how well a product satisfies the need of the consumers.
- Good manufacturing practice (GMP) is a system for ensuring that products are consistently produced and controlled according to quality standards. It is designed to minimize the risks involved in any pharmaceutical production that cannot be eliminated through testing the final product.
- Contamination controls:
  - Gowning, hand gloves, IPA (isopropyl alcohol)
  - Equipment sterilization at 121°C
  - HEPA filters (high efficiency particulate air filter) with 99.97% efficiency
  - Environment monitoring with SCDA media (Soyabean Casein Digest Agar) for sterility testing of microorganisms
  - Fumigation



Roles of quality assurance:

- Procurement of raw material and packaging
- Approval of vendors
- Auditing (internal audits of other facilities)
- Recording and documentation of processes
- Training the labour in manufacturing
- Facility and equipment designing
- Cleaning validation: method, process, duration, automation
- Line clearance
- Reviewing and sampling at every stage of production
- Market complaints and recall
- Pharmacovigilance: assessment and prevention of adverse effects
- Process validation and detailed standard operation procedures (SOPs)
- Equipment qualification [figure 6]

Process validation and equipment qualification ensures consistent quality in the products!

Figure 6. Equipment qualification flow chart

- The Central Drugs Standard Control Organisation (CDSCO) is India's national regulatory body for cosmetics, pharmaceuticals and medical devices.
- SOPs (standard operating procedures):
  - An SOP is a tested, verified, approved, and documented way of executing operations that form the pharmaceutical industry's basis. It provides step-by-step guidance for the personnel to perform a specific process. [figure 7]

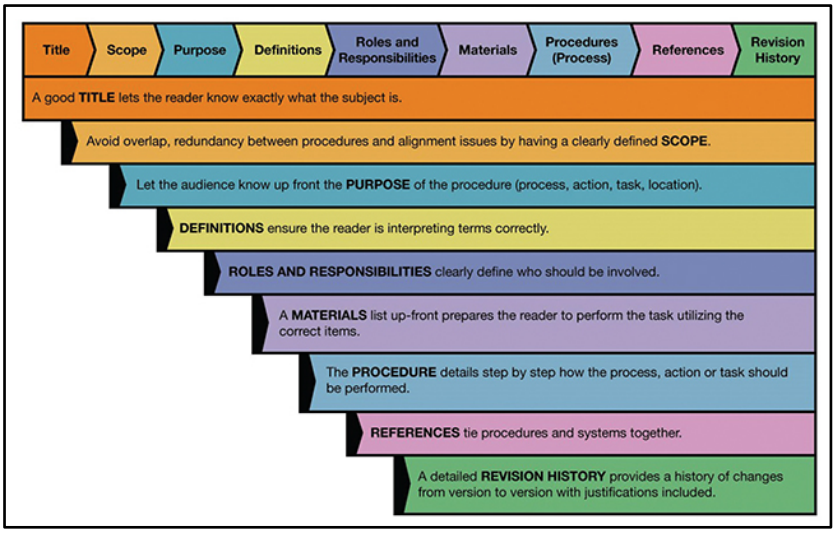


Figure 7. Basic elements of an SOP

4. R&D: Analytical chemistry lab

Mentor: Dr. Dipen Soni

Visited the analytical chemistry lab, learned about the equipment present, and observed an ELISA test.

- ELISA (Enzyme-linked immunosorbent assay) helps understand antigen-antibody interactions.

- An antibody is attached to a polystyrene plate which is a solid surface and is attracted or has an affinity towards bacteria, other antibodies and hormones.
- A microtiter coated with antigen is filled with this antigen-antibody mixture after which free antibodies are removed by washing.
- A second antibody specific to primary antibody is added which is usually conjugated with an enzyme.
- Free enzyme-linked secondary antibodies are removed by washing the plate.
- Finally, the substrate is added. The substrate is converted by the enzyme to form a coloured product, which can be measured by spectrophotometry.

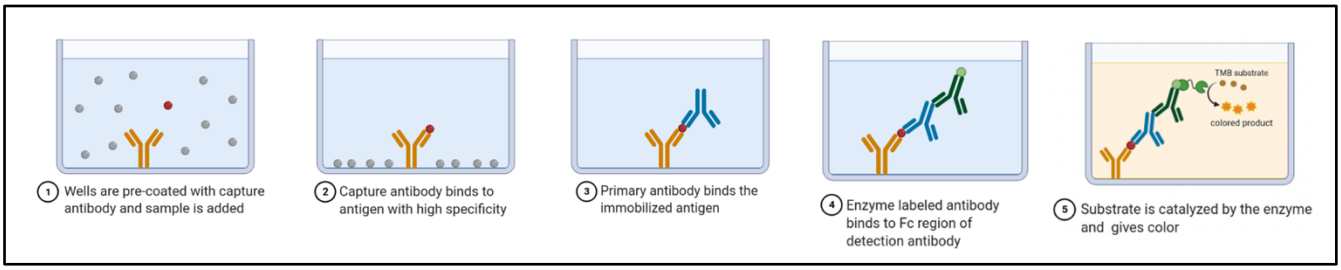


Figure 8. ELISA test

5. Quality control

Mentors: Dr Manish Gautam, Mr. Krunal Patel, and Mr. Pratik Ogale

- The objective of quality control is to test the drugs in various stages of production, verifying that they are able to proceed to the next stage in accordance with the regulations and specifications required for consumption.

Learned about the types of vaccines, pneumonia, as well as observed capillary electrophoresis.

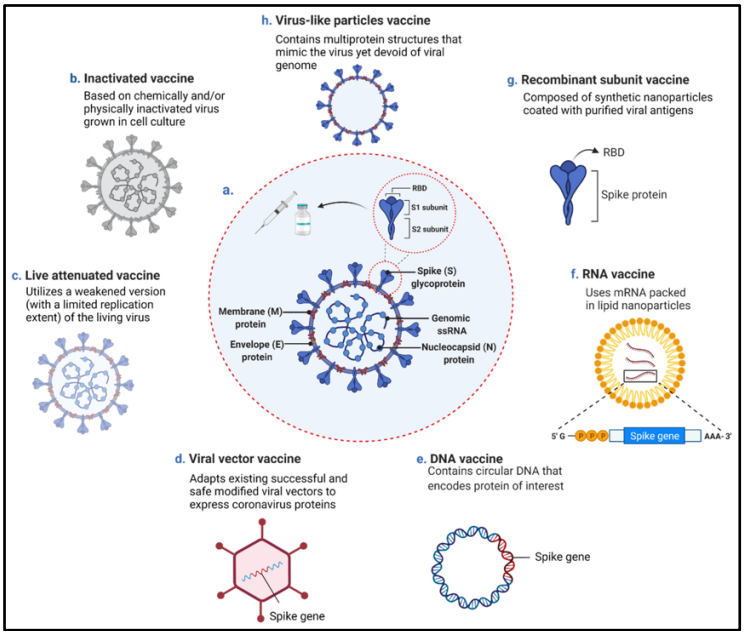


Figure 9. Types of vaccines

- Pneumonia:
  - Pneumonia is an infection of the lungs that may be caused by bacteria, viruses, or fungi. The infection causes the lungs' air sacs (alveoli) to become inflamed and fill up with fluid or pus. Pneumonia limits the oxygen capacity in the lungs, thereby making breathing painful. This can be observed with a lower SPO_2 level on an oximeter.
  - Bronchoalveolar lavage (BAL) is a diagnostic method of the lower respiratory system in which a bronchoscope is passed through the mouth or nose into an appropriate airway in the lungs, with a measured amount of fluid introduced and then collected for examination.
  - Pneumococcal Polysaccharide Conjugate Vaccine (Pneumosil) is used to protect infants, young children, and adults against disease caused by the bacterium Streptococcus pneumoniae (pneumococcus). It contains purified capsular polysaccharide of pneumococcal serotypes conjugated to a carrier protein.

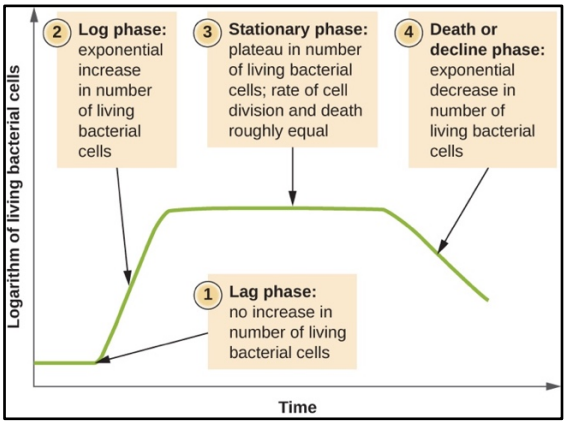


Figure 10. Bacterial growth curve

SDS – PAGE (Sodium dodecyl sulfate – polyacrylamide gel electrophoresis)	CE – SDS (Capillary electrophoresis – Sodium dodecyl sulfate)
They are used to obtain high resolution separation of complex mixtures of proteins based on molecular weight. The principle in both techniques is the same: during sample preparation, the samples are heated with an excess of SDS to denature the proteins. The addition of reducing agents cleaves disulfide bonds. Upon denaturation, the randomly coiled polypeptide chains open up.	
Limitations: Use of toxic reagents, higher chance of human error, and lower quantifiability of proteins.	Advantages: Increased automation, enhanced resolution, and more accurate quantification of proteins.

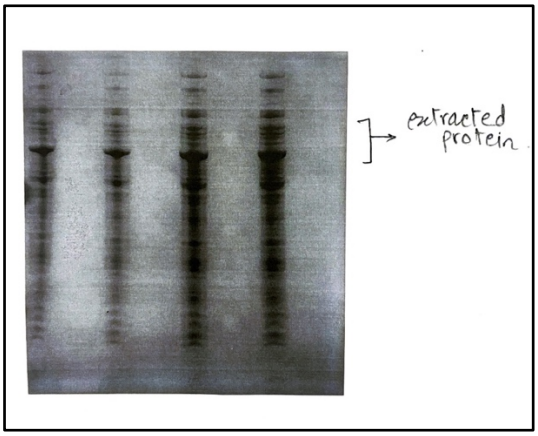


Figure 11. Result of SDS-PAGE