



Biobanking Quality System- Why and How?

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Aim

- To formulate and implement Biobank Quality Assurance systems that fulfil demands on scientific quality and minimal risks and that are regularly audited and improved.



- A Quality Assurance (QA) system is a continuous process that aims at measuring, evaluating and continuously re-evaluating the quality and when required, changing and improving the quality. In order to do so:
- The quality assurance work must have an aim (plan of activity and schedule for work)
- All the employees of a biobank must be involved in the QA system
- Results must be followed up, reported and analysed
- Corrective measures must be carried out based on the analyses



- The QA system must guarantee that the products (biological samples, health science information from e.g. health data registries, medical records or questionnaires and data from analyses of samples) that are managed have the quality that corresponds to the intended use.
- The QA should cover all activities in a biobank like purchasing of material, selecting contractors, licensing the staff to perform different assignments, collecting transporting and storing of samples, handling of data until “the product” is delivered.
- It is important to be able to define the quality of the individual sample/piece of information and to ensure that all activities (including mistakes) of every sample/piece of information can be tracked down and documented.- Traceability!



Good Biobanking Practise

- Term coined by the Medical Biobank in Umeå to denote a QA system ensuring that biobank products have a documented (traceable) quality that corresponds to the intended use.



Good Biobanking Practise:

Major features



- Separate, readily identifiable quality manual.
- Identity, ownership and organisation must be unambiguously documented.
- Project management must be unambiguous, from the procedure for scientific review of requests for samples, over integrity-assured handling of samples and data to code-keeping and statistical analysis.
- Security systems for preventing samples being destroyed and for prevention of inappropriate access to samples or information linked to samples must be in place.
- Number and type of samples must be registered.
- Characterisation of usefulness of samples.
- Handling procedures to ensure maximal usefulness of samples.



There should be a system for reporting quality deviations-
including complaints from customers/scientists.

There should always be an action taken in response to a quality deviation. The aim is “*never to make the same mistake twice*”

As a preventive measure it is important with an open minded attitude towards other platforms and collaborators.



Networking of the Quality work

- Defining quality indicators and how they should be measured.
 - E.g. Biological standards
- It should be possible to measure quality in the same manner! Improvements over time? Benchmarking compared to others?
- In quality work "What you get is what you measure"- Measured quality indicators typically will improve, unmeasured ones will not change.
- Quality indicators should be relevant for the intended use of the biobank.



Networking of the Quality work

- Sharing of experiences- ”quality improvements without having had to experience the mistakes/disasters first”.
- Two very different types of Networking Standards:
 - Identical/Enforced: e.g. Quality indicators and how they should be measured.
 - Examples, e.g. Standard Operating Procedures found to work well. May freely be copied, modified or ignored- Local situations differ.



Standardising Procedures or Standardising Quality Indicators and how they are measured?

- Standardising the SOPs is not always a good idea.
 - Takes very long time and efforts to agree on details.
 - Stops development work/improvements. New SOPs that give even better quality will not be discovered.
- But common standards/quality indicator measurements will promote progress by enabling sharing/comparing results/experiences.



**World Health
Organization**

GLOBAL HPV LABNET



Towards global quality assurance and comparability of HPV DNA and HPV antibody measurements



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Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses

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International multicenter study of 10 laboratories active in HPV serology. No attempt at standardisation of protocols.

Several formats of VLP-based EIAs and neutralisation assays

Composition of the serum panel

- 1. Antibodies induced by infection: Monotypic sera of three major HPV types currently being considered for vaccination (HPV types 6, 16 and 18).**
- 2. Negative control serum sample from virginal women.**
- 3. Antibodies induced by vaccination with monovalent vaccines (HPV 11, 16 and 18).**
- 4. Pools of the monotypic sera.**

All samples coded and all laboratories anonymous.

All statistical analysis at external center, not involved in HPV diagnosis (National Institute of Biological Standards and Control, UK)



Results from HPV 16 immunoassays



	HPV serotype	Laboratory									
		1	2	3	4	5	6	7	8	9	10
NIB-01	16	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-03	6+11+16+18 #	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-04	Vaccine	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-05	Vaccine	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-07	Vaccine	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-08	16 #	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-09	6+11+16+18	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-10	Vaccine	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-12	Negative	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-13	18 #	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-14	Vaccine	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive
NIB-16	6+11 #	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive	Reactive



Intitally wide range of titres in HPV 16 immunoassays



		Range of titres
NIB-01	HPV 16 natural infection	100-640
NIB-03	Pool HPV 6+11+16+18 #	<100-640
NIB-04	Vaccine	100-2560
NIB-05	Vaccine	384-2560
NIB-07	Vaccine	400-2560
NIB-08	HPV 16 natural infection #	<20-160
NIB-09	Natural HPV 6+11+16+18	<20-80
NIB-10	Vaccine	1600-10240
NIB-12	Negative	-
NIB-13	HPV 18 natural infection #	-
NIB-14	Vaccine	5381-40960
NIB-16	HPV 6+11 natural infection #	-



**Including the same reference standard serum
in the assay resulted in low interlaboratory
variation- even without any attempts at assay
standardisation
(results given in PLL-calculated Units of HPV
antibody levels)**

Sample	Laboratory										Mean potency	%GCV	Neutralisation
	1	2	3	4	5	6	7	8*	9	10*			
08	0.24	0.45	0.37	0.37	0.54	0.50	0.42	0.40	0.68	0.74	0.43	33	0.4
09	0.09	0.54	0.41	0.27	0.36	0.29	0.16	0.10	-	0.30	0.24	91	0.3
03	0.07	1.10	0.20	0.22	0.29	0.26	0.11	0.25	0.19	0.26	0.22	111	0.2
04	4.93	6.70	4.04	5.13	4.45	5.21	6.71	9.95	8.24	3.26	5.91	35	16
05	2.05	4.03	3.91	3.32	2.35	3.11	3.67	1.79	4.63	2.20	3.06	39	4
07	4.14	3.88	5.06	4.79	5.12	4.15	6.19	6.65	7.24	2.92	5.13	25	54
10	16.44	18.10	16.28	16.54	12.76	10.20	29.99	17.40	24.23	3.03	17.2	37	102
14	24.68	53.85	74.21	40.07	26.14	55.02	91.51	25.03	59.30	3.13	45.10	63	870



Antibody Unit Standardisation



- **Simply relating measurements to the same International Standard greatly reduced interlaboratory variability.**
- **Definition of International Units will allow comparing HPV antibody levels between different studies and different vaccines**
(Currently reported as "milliMerck Units", "EIU", "titers", "OD reading", other "Units")
- **Meaningful definition of level of antibodies required for protection will require International Units.**
- **Helpful in development of second generation vaccines.**

- **Reference laboratories will need to be established, connected to the monitoring of the vaccination programs**
- **Seroepidemiology: a research tool in analytic epidemiology, likely to become increasingly important for upcoming HPV eradication (vaccination) programs**



HPV DNA standardisation

Global WHO collaborative effort-
29 laboratories worldwide, testing the same
samples.

J Clin Microbiol, 44,571, 2006

Assay variability much greater than expected (up
to 5 logarithms differences in sensitivity!! False positive
results common also in "expert" laboratories.)

The WHO Global reference lab (i.e. our lab) will issue
proficiency panels to enable global accreditation
standards.



Swedish National Biobanking Program

- Biobanks throughout Sweden committed to sharing quality work experiences. Everyone supposed to contribute but coordination at:
- Umeå (Hallmans): Process documentation/Manuals
- Malmö (Carlson): DNA
- Stockholm (Niste´r): Protein
- Uppsala (Botling): RNA



Material transfer agreement (MTA) checkpoints – pilot project

- Approval from Biobank group of experts and/or PI
- Approval of all parts of the MTA
- Approval from the ethical review board
- Approval from the data inspection board – protection of personal integrity in genome studies



DNA evaluation methods

The quality of DNA evaluated by:

- DNA yield

Pico Green measurements (if missing, A_{260})

- DNA purity (A_{260}/A_{280})
- Molecular weight analysed with agarose gel electrophoresis
- Amplification with PCR with increasing amount of DNA to reveal inhibiting factors
- Real-time PCR for functional DNA



Quality aspects on DNA. Sample retrieval and extraction



DNA extraction

To minimise risk due to sample mix-up, sample loss and inconsistent yield, buffy coats are divided in **two aliquots**, retaining half in the original tube available for later recovery if necessary.

- Average DNA yield from a divided buffy coat sample: 120 μg
- No effect on yield up to 10 times of freeze-thaw cycles
- No effect on purity after repeated freeze-thaw cycles
- No effect on molecular weight after repeated freeze-thaw cycles
- No negative and inhibiting factors after repeated freeze-thaw cycles affecting PCR amplification



Storage of DNA aliquots

After concentration measurement the homogenous DNA sample is divided into 4 different cryo tubes.

- 2 μg is transferred to one tube for long-time banking.
- A working solution (300 μl x 100 $\mu\text{g}/\text{ml}$) is stored in a second tube.
- The remaining sample at its original concentration is split into two aliquots.
- This distribution is an important safety precaution if sample mix-up or contamination occurs at a downstream handling.



Whole Genome Amplification (WGA)

Routines for amplification of samples with scarce amount of DNA have been **validated** by comparison of amplified material with original sample. Following WGA, functional DNA concentration is determined by realtime PCR. This method is very useful for the maintenance of the biobank.



Shipment of DNA

- Eppendorf tubes and microtiter plates for transportation of DNA to the different sites for analysis.
- DNA is either wet or dry.
- Dispersion of DNA into 96-well microtiter plates and evaporation at room temperature before sealing is preferred. Simplifies the transportation of samples
- Wet DNA transportation demands dry ice which greatly increases the costs.



Information following DNA samples – delivery agreement

To secure the samples and facilitate the downstream applications, a delivery agreement must follow the shipment.



For shipment - needed additional information

Institution sending and receiving the samples.

Date of sending and receiving shipment.

Researcher responsible for sending the shipment and address, telephone number and mail address.

Researcher and/or co-worker receiving the samples, address, telephone number and mail address.

Project name and number.

Number of samples, tubes or plates

List of tubes and/or plates and samples (preferably XL compatible file)

Instructions concerning what to do with remaining samples after analysis

Information about how samples and data must be handled according to the Swedish Personal Information Act.



For individual samples the following information must be available

For samples in plates

- Plate identification
- Sample identification number
- Sample position in plate
- Original DNA concentration
- Volume added in well
- Total amount of DNA in well
- DNA extraction method
- Remaining sample after analysis



For samples in tubes

- Sample and tube id.
- Position in box
- Box id.
- Original DNA concentration
- Volume added in tube
- Total amount of DNA in tube
- DNA extraction method
- Remaining sample after analysis



Additional recommended information

- DNA extraction has been performed **randomised** between cases and controls
- Cases and controls are distributed in the **same plate**
- DNA controls are distributed in the plate and **blinded** for the staff performing the analysis
- Duplicate samples should be randomly placed within each series with identities blinded for the analytical staff.
- Some wells should be left empty for negative or other controls needed for analysis performance
- If any discrepancy from standard sample handling has occurred



Quality aspects on plasma sample handling



Plasma is stored at -80°C after pipetting from fractionated whole blood and transferred to cryo tubes.

10 ml whole blood renders plasma, divided in 3 parts with approx. 1,5 ml each.

- Diminishing amount of material is needed for analysis of protein and other metabolites in serum and plasma



Quality tests plasma, (thawing)

2

- Tests were performed where thawing at +4°C was compared with RT and 37°C. No significant changes could be seen on the levels of ferritin, iron, UIBC, albumin, leptin or adiponectin.
- Specifically for vWF 37°C is better but not for other purposes (?)
- A comparison of plasma samples thawed at +4°C and 37°C was performed using Seldi and scanning the protein content ranging from 2500 D to 15000 D with no indication of protein degradation.



Rules for analysis platforms and laboratories

- The analysis platform has no right to keep remaining sample after analysis is performed
- The result of the analysis must be transferred to PI and/or data management service centre
- The information on cases and controls is accessible through PI and/or data management service centre
- **Analysis must be totally unbiased** concerning cases and controls



Quality aspects on data administration

Increasing amount of data following samples, questionnaires and results of analysis demands a fully **functional internal data network system**:

- Optimal operational security
- A fast and accurate up-dating mode
- A smoothness in exchanging information within the biobank group
- A system that facilitates standardised study designs.
- A reliable back-up system and physical protection



For the future

- Evaluation of new storage techniques e.g. for automation
- Web-based quality system opened up for licenced users
- Education activities within the quality control program
- Development of a data administrative system increasing speed, accuracy and security