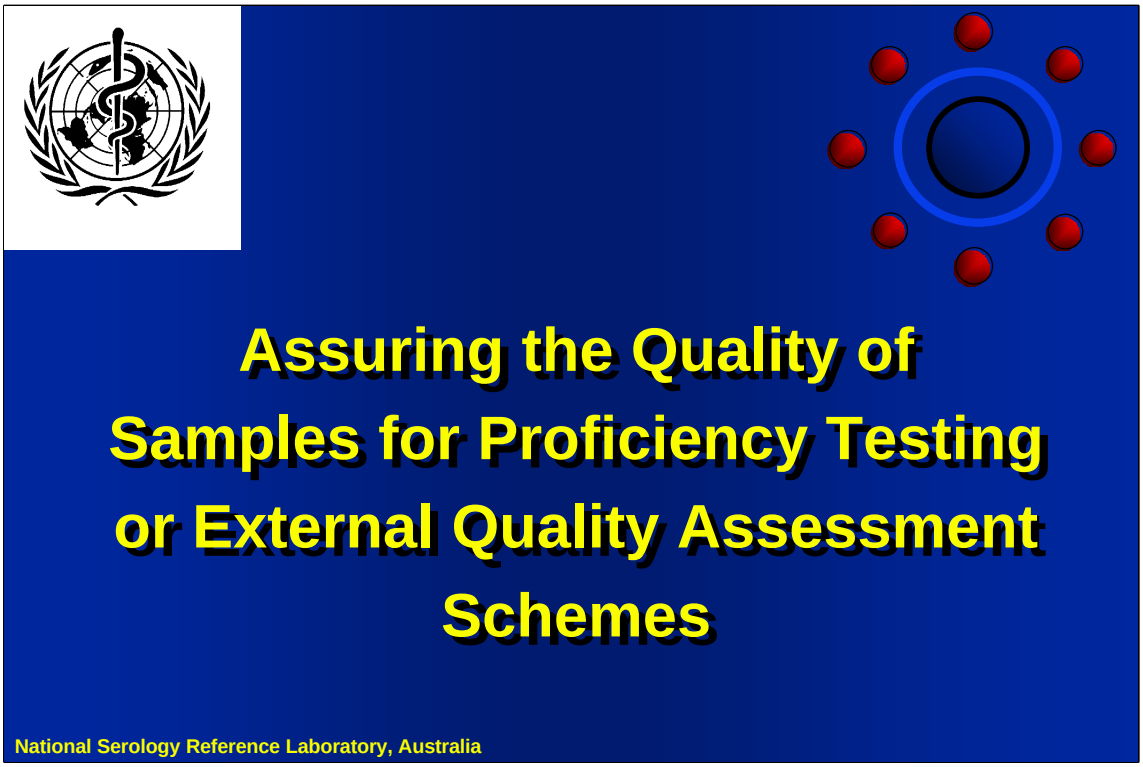


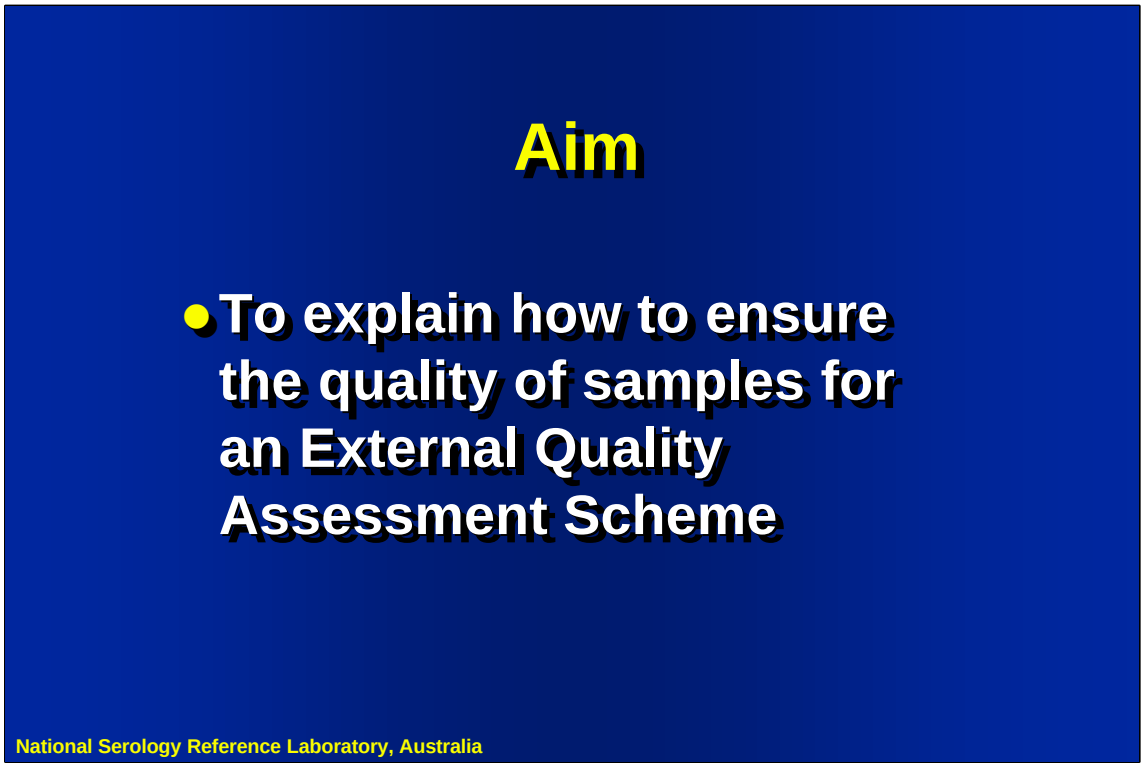
Terminology

External quality assessment is a method for assessing the entire laboratory testing process including the quality of results. Usually quality assessment involves an external evaluation of a laboratory's performance in panels of samples prepared by a reference facility. Quality assessment is **not** a substitute for laboratory quality management which should include a series of quality assurance measures.

External Quality Assessment Schemes (EQAS) are programmes orchestrated by a central (or national) reference laboratory which distributes, analyses and reports on EQAS panels and their results. If the scheme is a nationally based scheme it is called a national external quality assessment scheme or NEQAS.

Quality EQAS Sample: A sample that is included in an EQAS panel which will give the expected results in the most cases possible.

Proficiency Testing and EQAS are closely allied. Differences are explained in Libeer *et. al*., 1996.



Aim

Going to explain how to ensure the quality of samples for EQAS. Because,if we use good quality samples then the participants will get useful results.

By “Quality samples” I mean, samples that are produced in a consistent and traceable way.

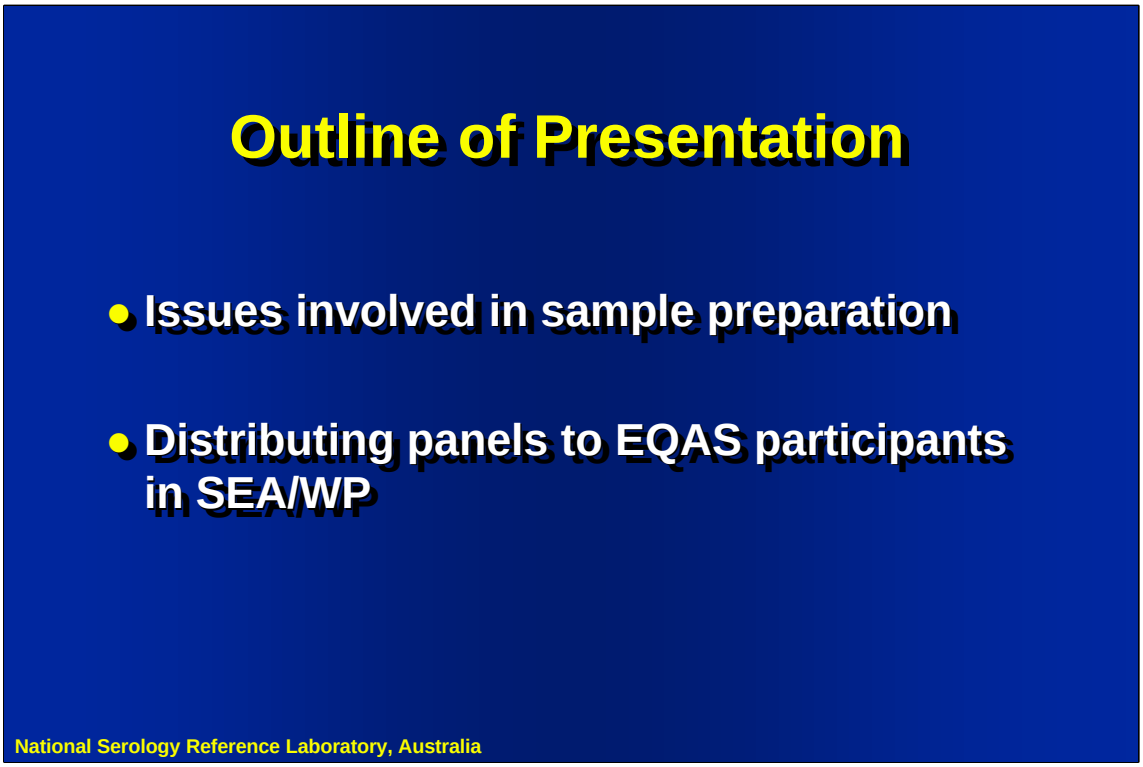
In order to have good quality samples for EQAS we need to:

•acquire and select good quality samples

•store the samples properly

•prepare the samples with utmost care

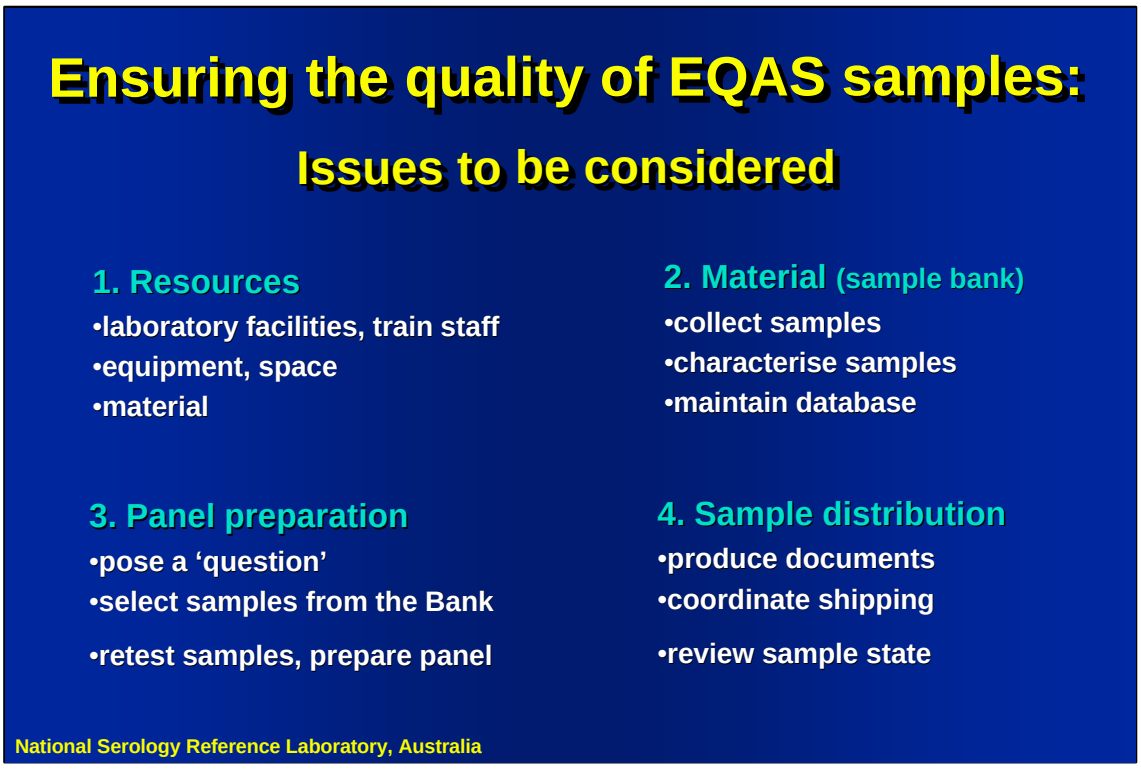
•EQAS panels are distributed and arrive in pristine condition



Outline of presentation

- I'm going to discuss the steps to produce EQAS samples. Issues for each step will be discussed.

- At the end of the talk I will relate some of the experiences of the NRL after distributing samples to South-East Asia/Western Pacific.



Ensuring the quality of EQAS samples:

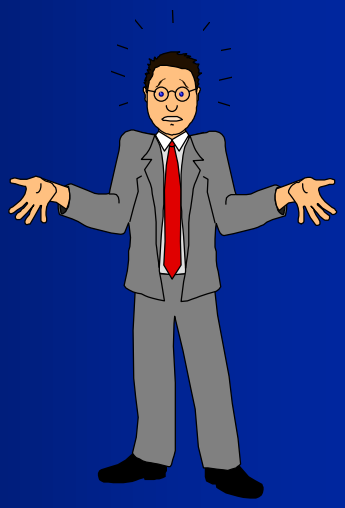
I'm going to discuss 4 steps involved in producing good quality samples.

To produce good quality samples you need to:

1. Designate resources- such as time and money
2. Develop a sample bank where samples can be stored and found again easily
3. Good procedures for preparing samples for the EQAS panels
4. Distribute panels for EQAS ensuring **transport requirements and regulations** are followed. Remember that this is the time where the samples are most vulnerable and they are no longer in your control.

Preparing quality EQAS samples

- Try and decide:
  - Who?
  - What?
  - Where?
  - When?
  - Why?
  - How?



National Serology Reference Laboratory, Australia

So we want to produce good quality samples…….

A good way to ensure that good quality samples are produced is to ask questions for each step in sample production.

When all the questions are answered this will lead to quality samples.

Take sample distribution for example:

•Who will be trained to pack and ship the samples?

•What paperwork will we include with the samples?

•What paperwork do we need to fill out to get samples in to another country?

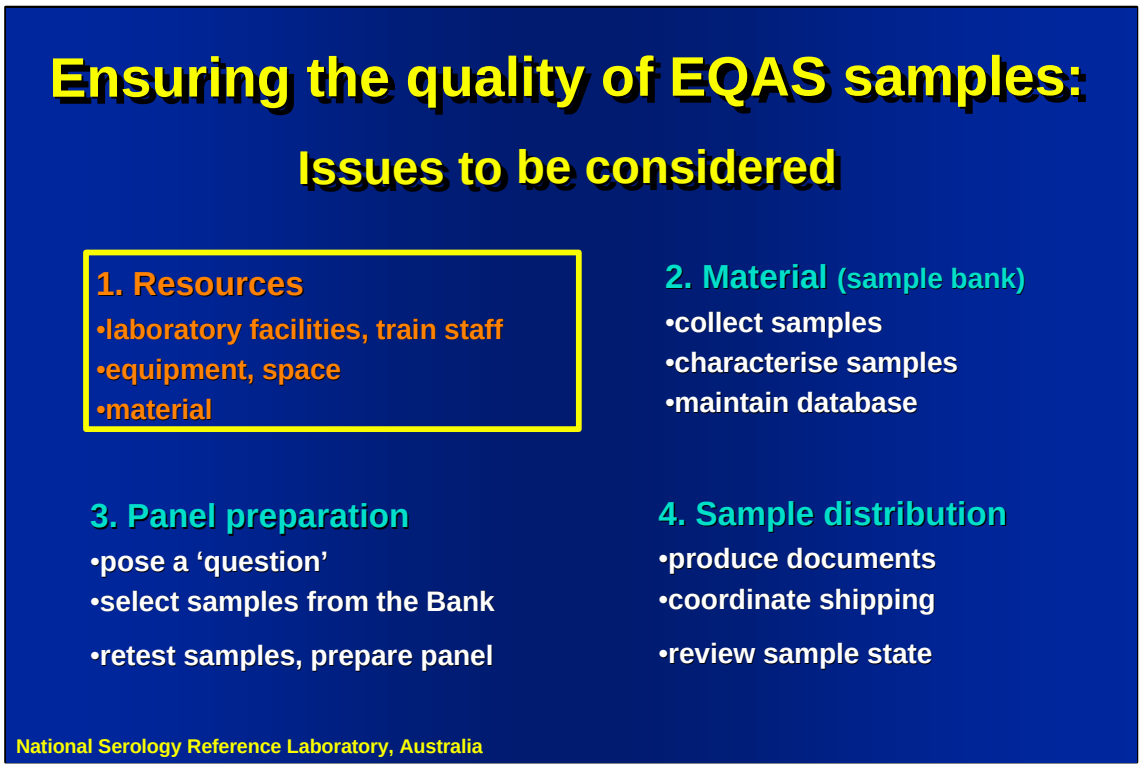
•What company will we use to ship the samples to participating laboratories?

•Where are the shipments to be distributed and how will we know they arrived?

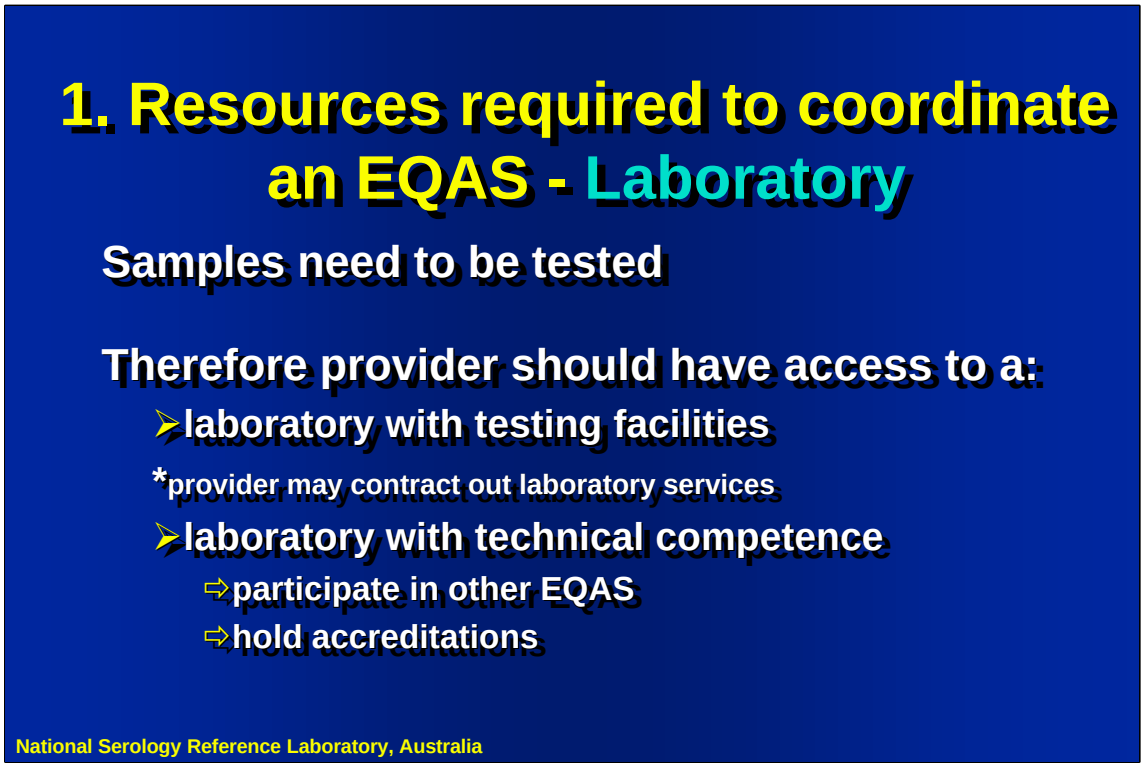
•When do the samples need to be shipped out?

•Why does attention need to be paid to distribution and its various requirements?

•How will samples be packaged for shipping?



1. Designate resources- samples time, money, equipment, space

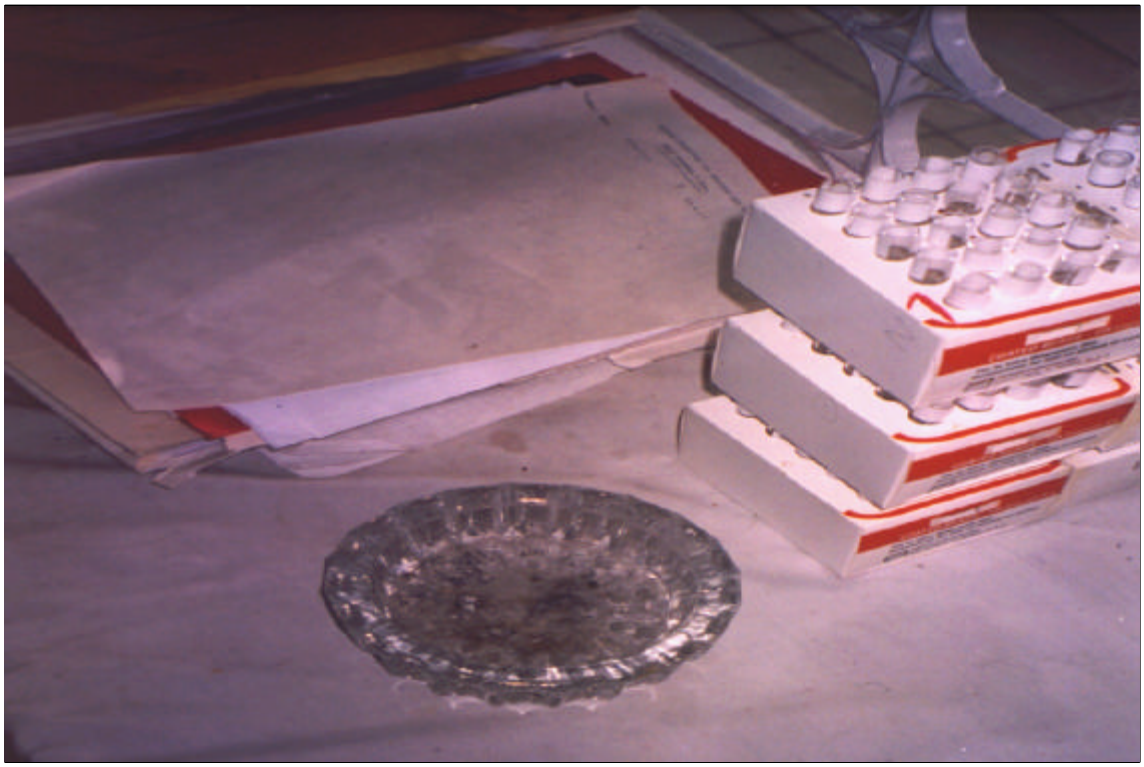


1. Resources

- Remember that samples need to be tested:
  - 1) before going in to sample bank, i.e., determine their disease status and reactivity to tests;
  - 2) after their removal from sample bank and before distribution to ensure that the reactivity has not altered during storage

- **It is important that laboratories participating in an EQAS have confidence in the quality of the samples.** So the EQAS provider must have access to a testing laboratory with technically competent staff. This will ensure that samples for the EQAS are of the best possible quality.

- Where available, the laboratory doing the testing for the EQAS provider should participate in an international scheme for quality assessment
- Ideally, the provider should aim to become accredited according to international guidelines (e.g. ISO 17025).



The laboratory that performs testing for EQAS samples should be of sufficient quality to ensure the results are accurate.

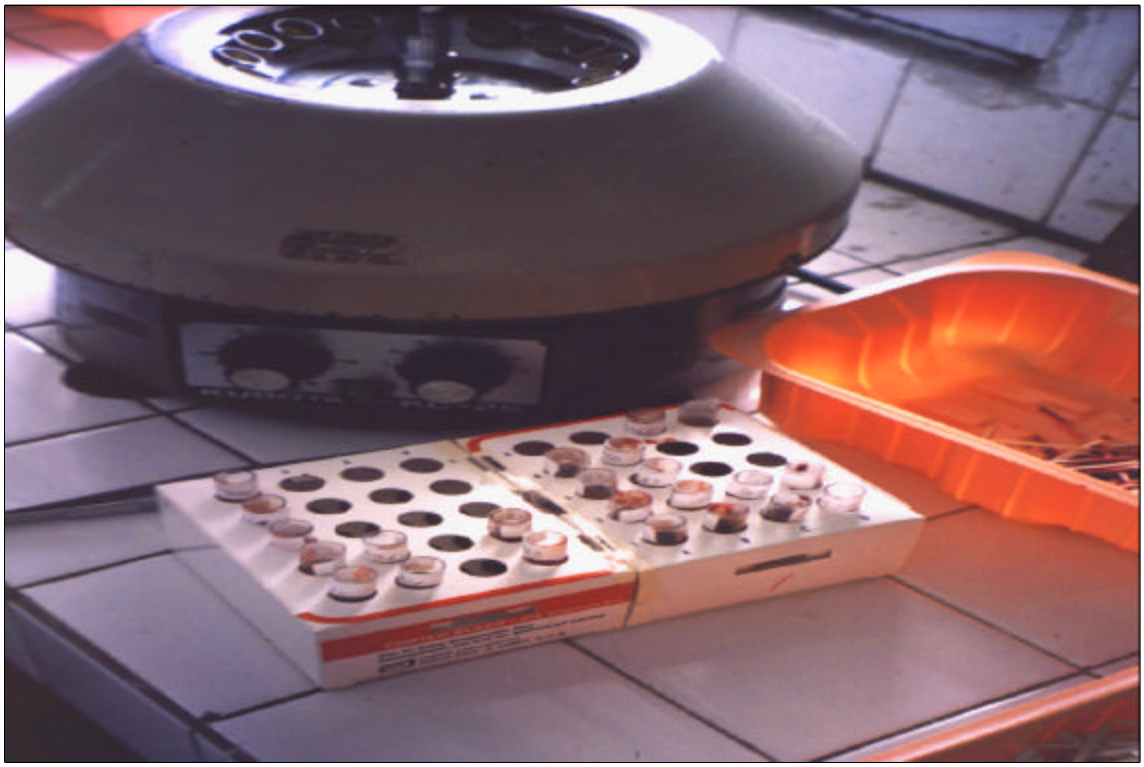
Problems with laboratory practices:

•Ash-tray next to samples with open tubes - presumably someone smokes while they work. *Putting something in their mouth*.

•Samples with no lids left next to paperwork.

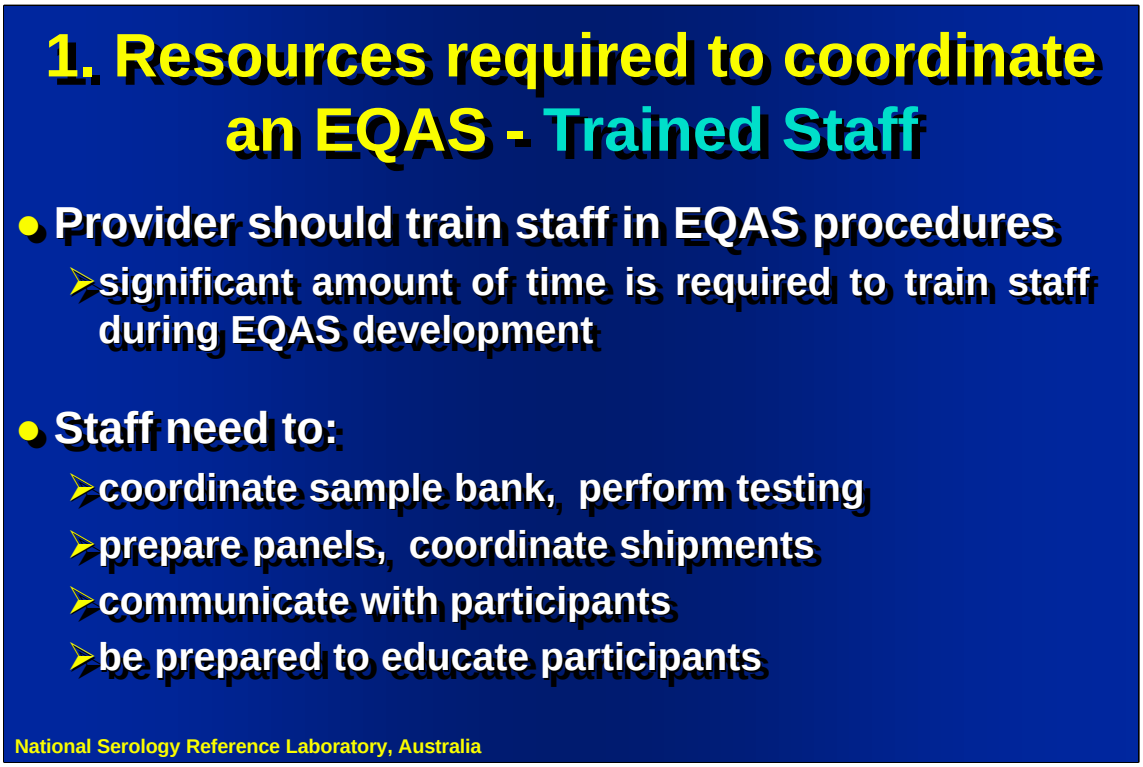
Same story:

This looks like a Tea-room but this is a laboratory. Someone is storing food and drink containers on the work bench.



Break up the talk a bit. I have been talking about ensuring the quality of the EQAS samples and the resources required to run an EQAS.

These specimen tubes are open and should not be used anyway but for EQAS you should ideally have polypropylene storage containers with screw cap lids and rubber rims

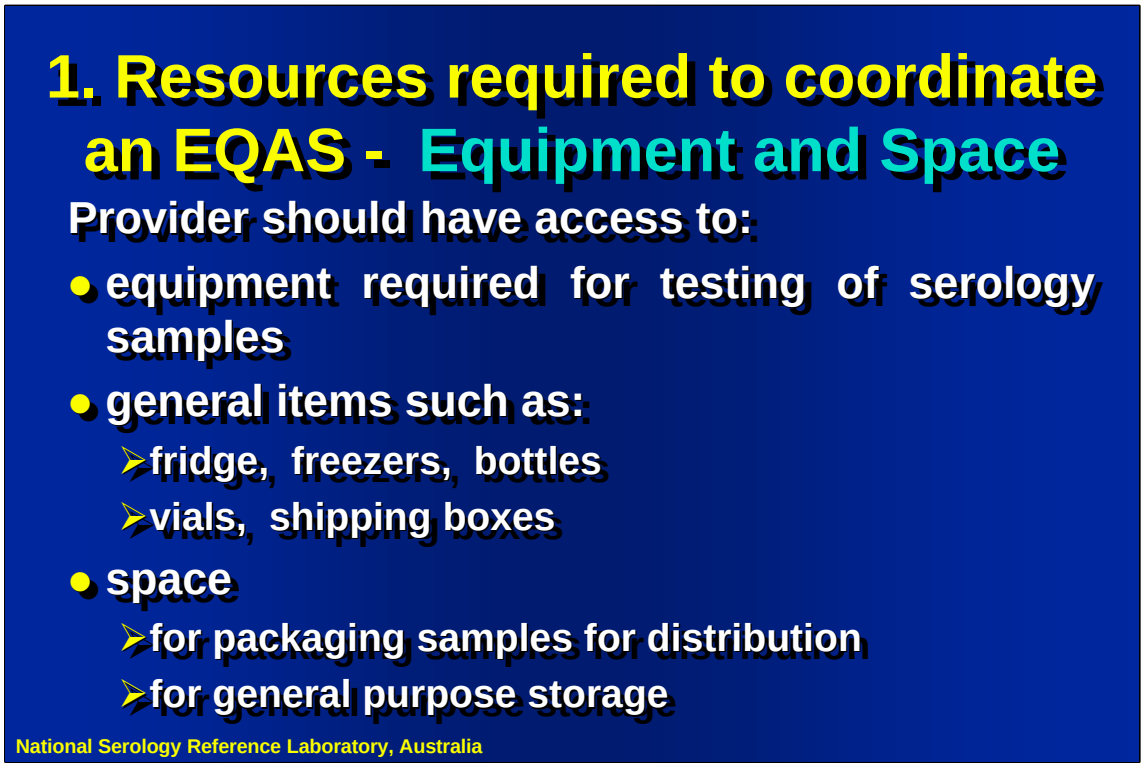


1. Resources

The EQAS provider should have staff specifically trained in providing EQAS

Good quality samples can only be produced if the staff are able to co-ordinate a sample bank and prepare panels and coordinate shipments.

Make sure that the time to train all the EQAS staff is invested to ensure the quality of the samples.



1. Resources

There are obviously other (Physical) resources that are required to produce good quality samples

The EQAS provider should have access to equipment and space

•The provider will need equipment for performing various diagnostic HIV assays - **pipettes, incubators, plate washers and plate readers**.

•The following general items are also required:

fridge, freezers, bottles

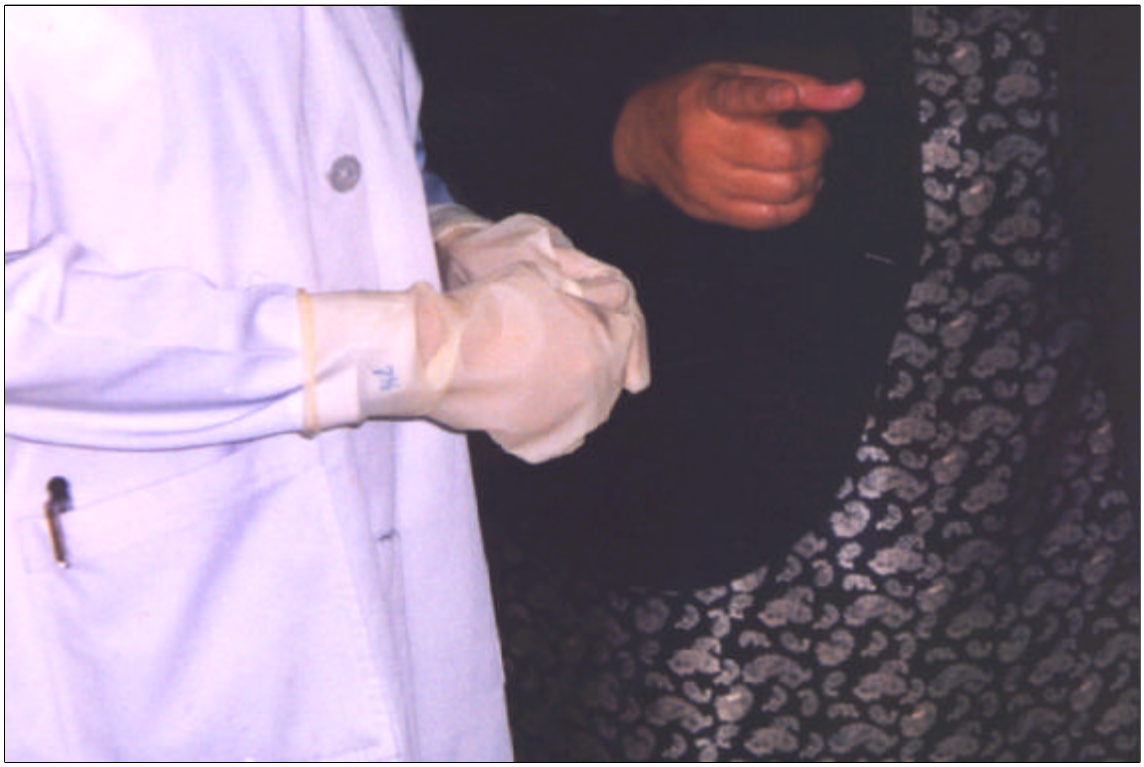
vials, shipping boxes

•**Space** will also be required for sample testing and packing of panels for distribution

•Also need storage space for vials and packaging materials

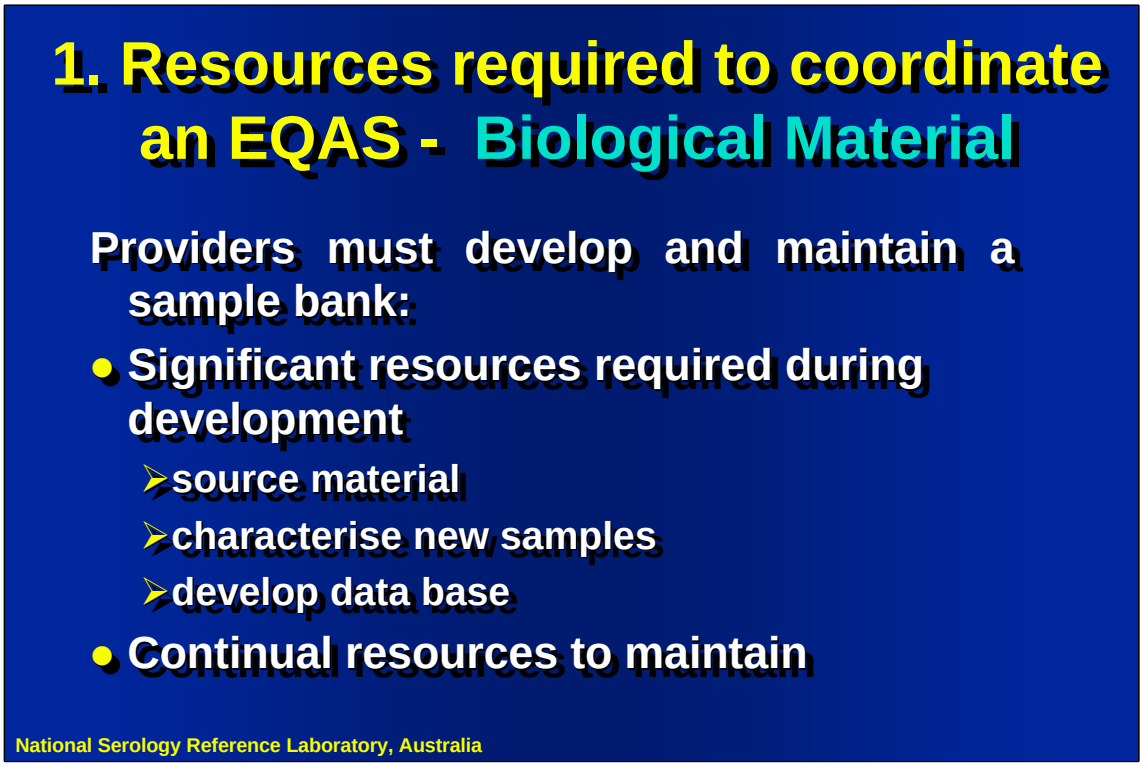
•**Ideally need a computer with a printer and a photocopier** to generate documents such as cover letters and result forms

•Biohazard hood for handling infectious material



Make sure that what resources you do use are appropriate.

Pay attention to detail- such as buy gloves that are the correct fit for your staff.



1. Biological Material

The EQAS provider should have access to samples of high quality and quantity. This will ensure that the EQAS provider can **maintain a delivery schedule and provide a range of materials to the participants.**

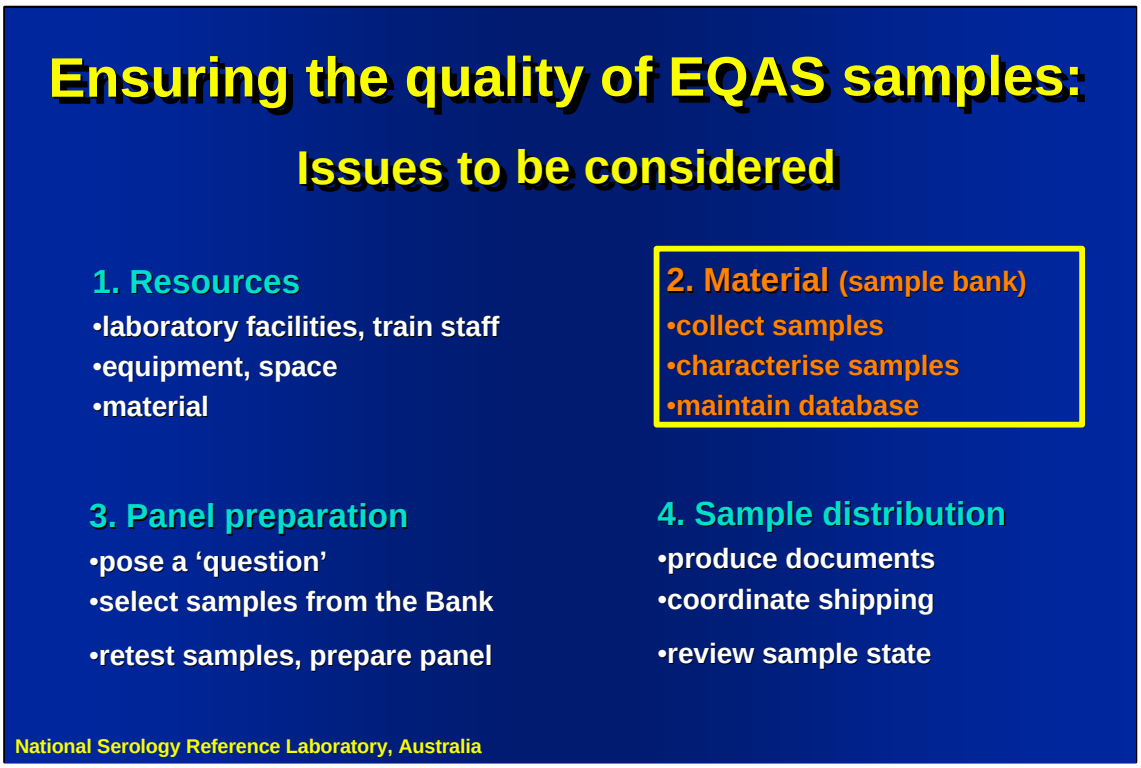
•A lot of time needs to be spent obtaining samples before the first EQAS panel is distributed.

•The samples used in an EQAS are a critical element of the scheme and a significant amount of time should be spent developing a sample bank to store the samples.

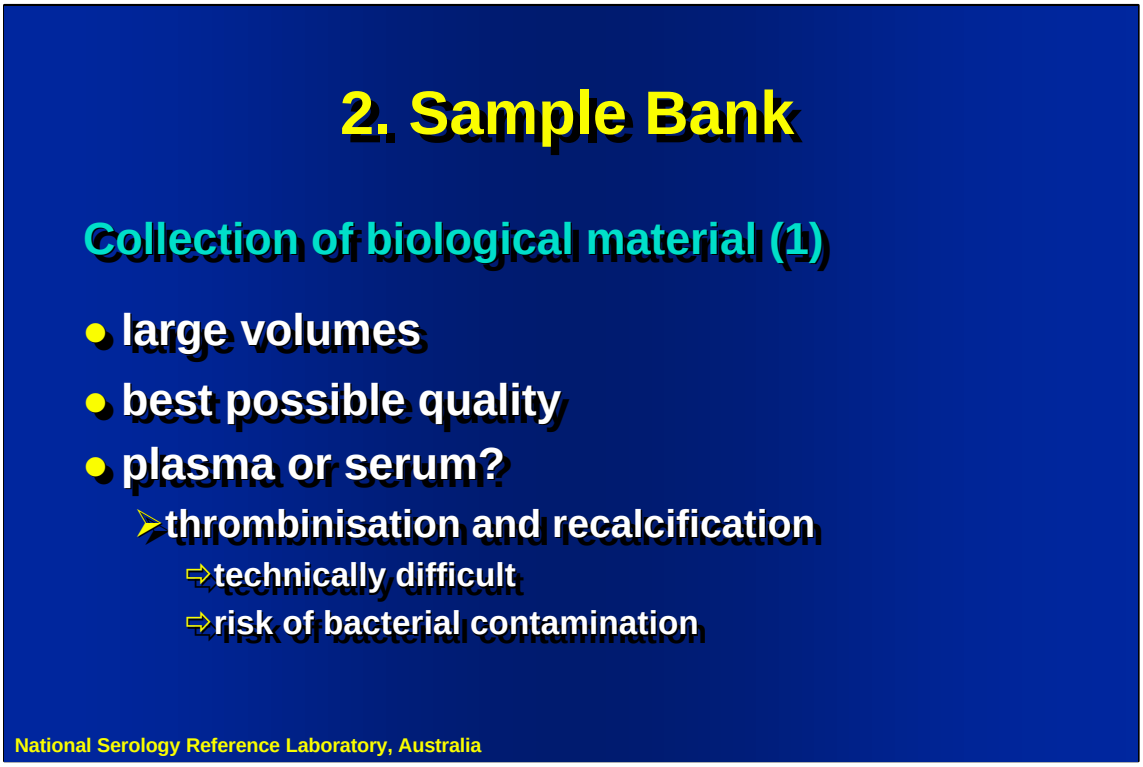
•A sample bank is

- •a freezer to keep the samples below -20ºC
- •a system for identifying and finding the samples.
- •Database to store all the information about each sample.

•It is important to remember to **Keep the database up to date!!.**



2. Material (or the samples) and the Sample Bank for storing the samples



2. Sample Bank

When collecting material for use in EQAS panels consider that large volumes are required -blood service donations best for acquiring large volumes

The volume of sample you need will depend on the number of participants and the type of testing they conduct. In most cases 0.5ml of each sample should be enough for each participant.

Remember to allow extra volume for testing the sample before distribution, and for volume lost when spinning and aliquotting samples. Our experience - at least an additional 40% to the volume required to produce the panel samples should be added.

•The samples introduced into sample bank should be of **best possible quality**. Samples should not have been through multiple freeze/thaw cycles or be heavily haemolysed or lipaemic.

•**Need to decide whether to use serum or plasma?** will depend on the diagnostic kits used in the area. (Most diagnostic kits use either plasma or serum.)

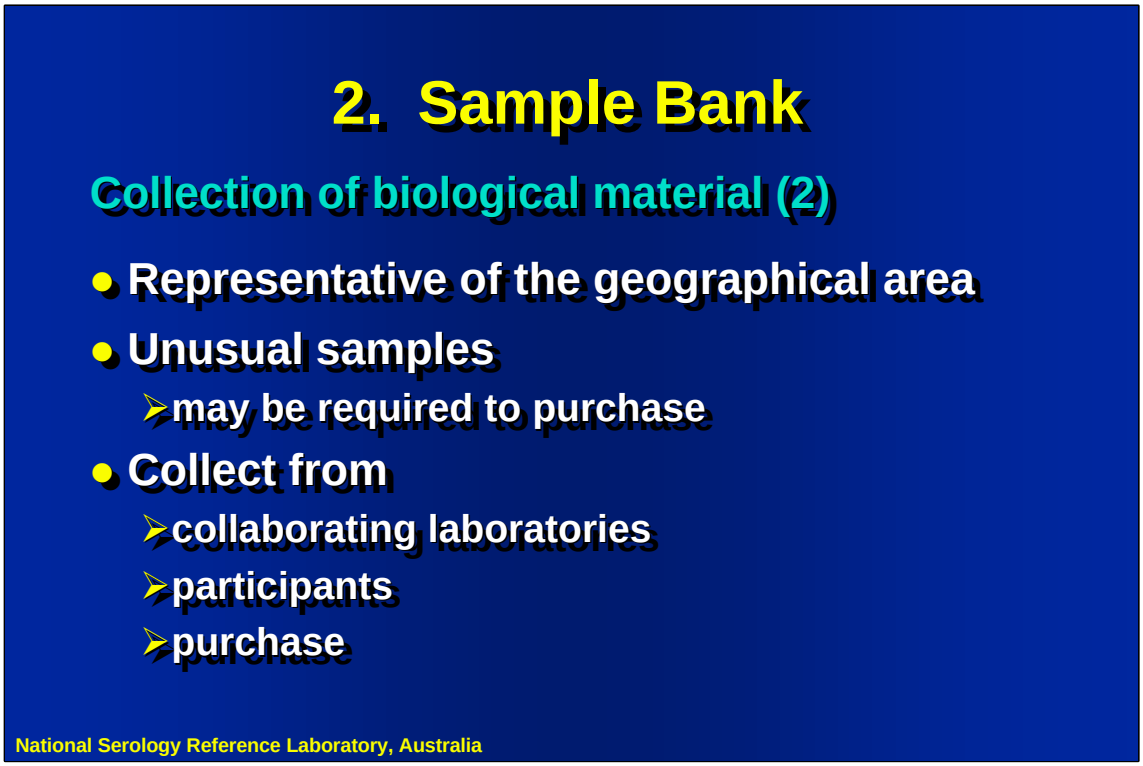
•Also depend on the volume required - blood service donations provide large volumes and blood donations are plasma.

Can convert plasma to serum. Two methods are available: **thrombinisation and recalcification**.

These methods are **technically difficult and increase the risk of bacterial contamination**, and handling involves safety issues.

Of the two methods **thrombinisation is preferred** as micro-clots are less likely to form during storage and a “cleaner” product is obtained. Excessive recalcification should be avoided as this adversely affects some assays, such as gelatin particle agglutination.

•Overall, the aim of an EQAS programme is to assess a laboratory’s testing process. Testing either serum or plasma samples will achieve this aim. If plasma is carefully handled, centrifuged prior to



2. Sample Bank

To provide quality samples in an EQAS, the samples used should be representative of types of HIV viruses circulating in the region and of the different stages in HIV infection.

For example, an EQAS for anti-HIV testing in Central Africa should contain HIV-2 and a range of subtypes that are predominant in the area.

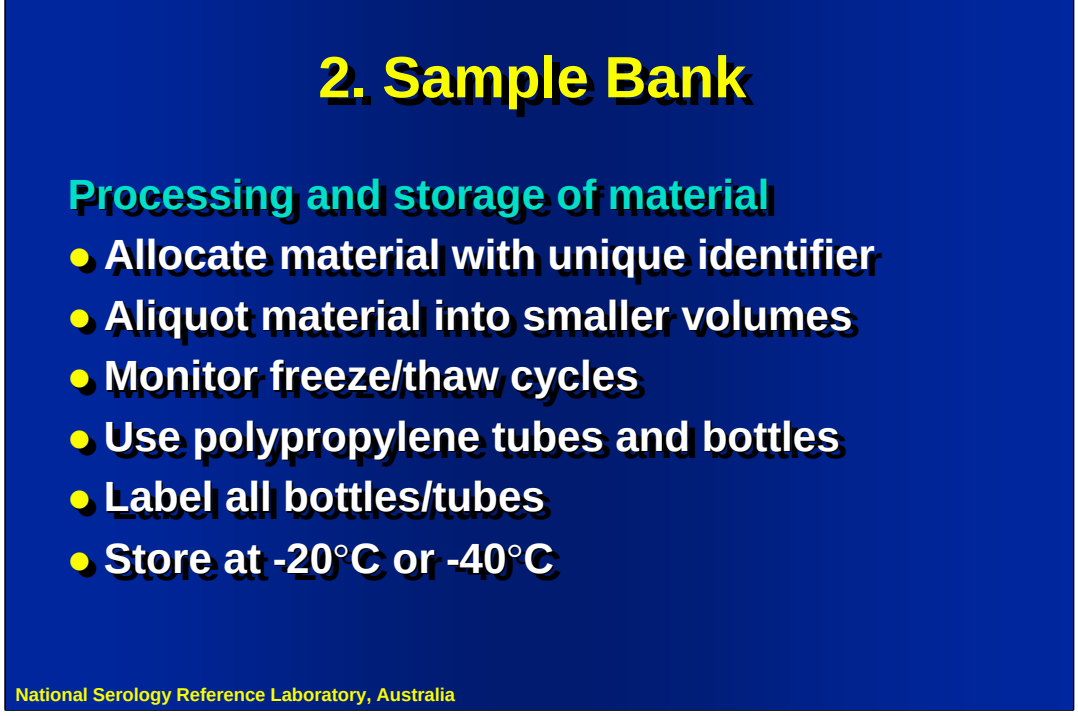
•**The samples you use** should be from different stages in HIV infection. However, this is not always possible. For example weakly positive material from early seroconversion is rarely available and large volumes of material should not be taken from AIDS patients.

• **Once an EQAS has been coordinated for a few years, may want to include more challenging samples** e.g. different subtypes, dually infected samples

•More challenging samples are often difficult to source and may need to be purchased from a commercial supplier. If purchasing samples, ask for a small volume of sample for testing, to ensure it meets your requirements, before purchasing larger volumes.

• **Collect samples by networking with participating or collaborating laboratories** and asking them to contribute specimens.

•**Where large volumes are required, liaison with blood services is important.**



2. Develop Sample Bank

To ensure the quality of the samples is maintained, samples should be processed and stored in a consistent way.

As soon as material is brought into the EQAS providers laboratory, give each sample a unique identifier. This is usually a code number that will be used for the life of the material.

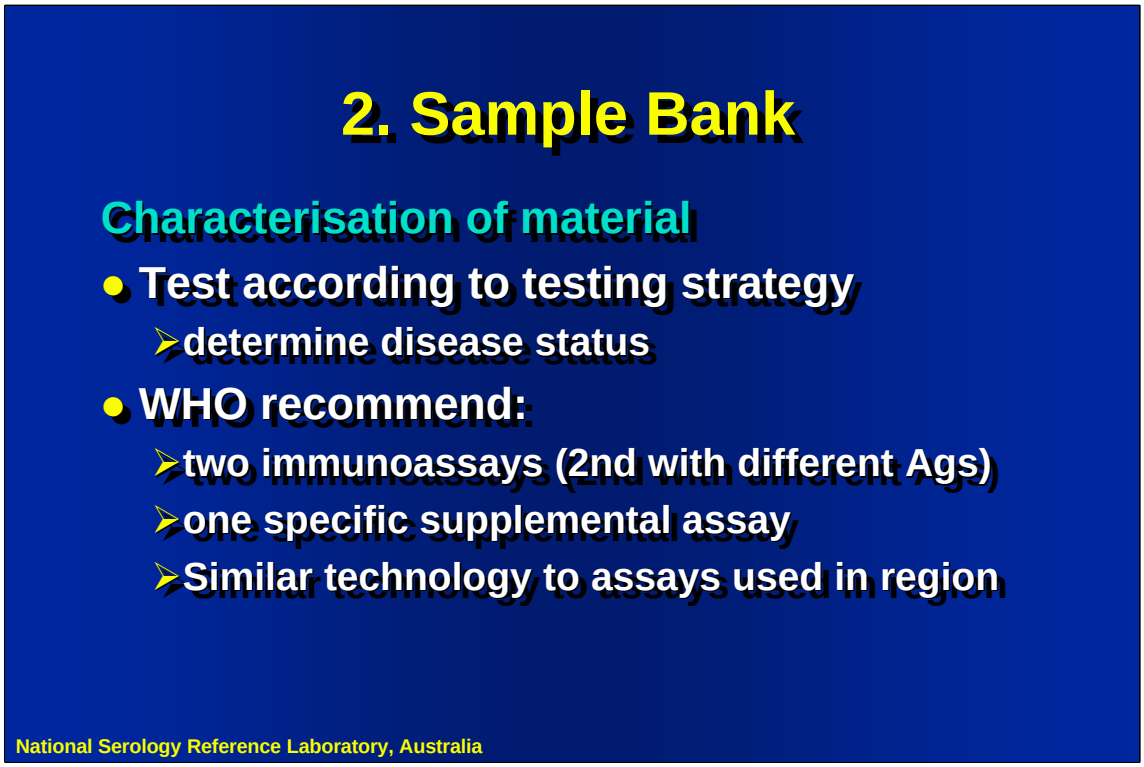
•**Large volumes of samples should be aliquotted into smaller volumes** sufficient for one EQAS distribution. Remember to include extra volume for testing and loss due to spinning and aliquotting.

•Aliquotting into smaller volumes will cut down on the number of freeze/thaw cycles. Ideally, samples should NOT be subjected to >3 freeze/thaw cycles. Mark the lid each time the sample has been thawed. For a short time, samples are better stored at 4ºC.

•**Storage tubes and bottles must NOT be polystyrene or glass** as these containers may adsorb antibodies and alter the antibody level of the samples. We always to use polypropylene storage containers with screw cap lids and rubber rims.

•Sample bank samples should be stored -20°C or -40°C and appropriately labelled with permanent freezer markers or labels.

•Samples should be stored in appropriate storage boxes. In a humid climate, storage boxes should be plastic or stainless steel because cardboard boxes may encourage mould.



2. Sample Bank

***Before going into Sample Bank*, samples should be characterised so that the serostatus is clearly defined. This will allow providers to select appropriate material for EQAS panels as they are required.**

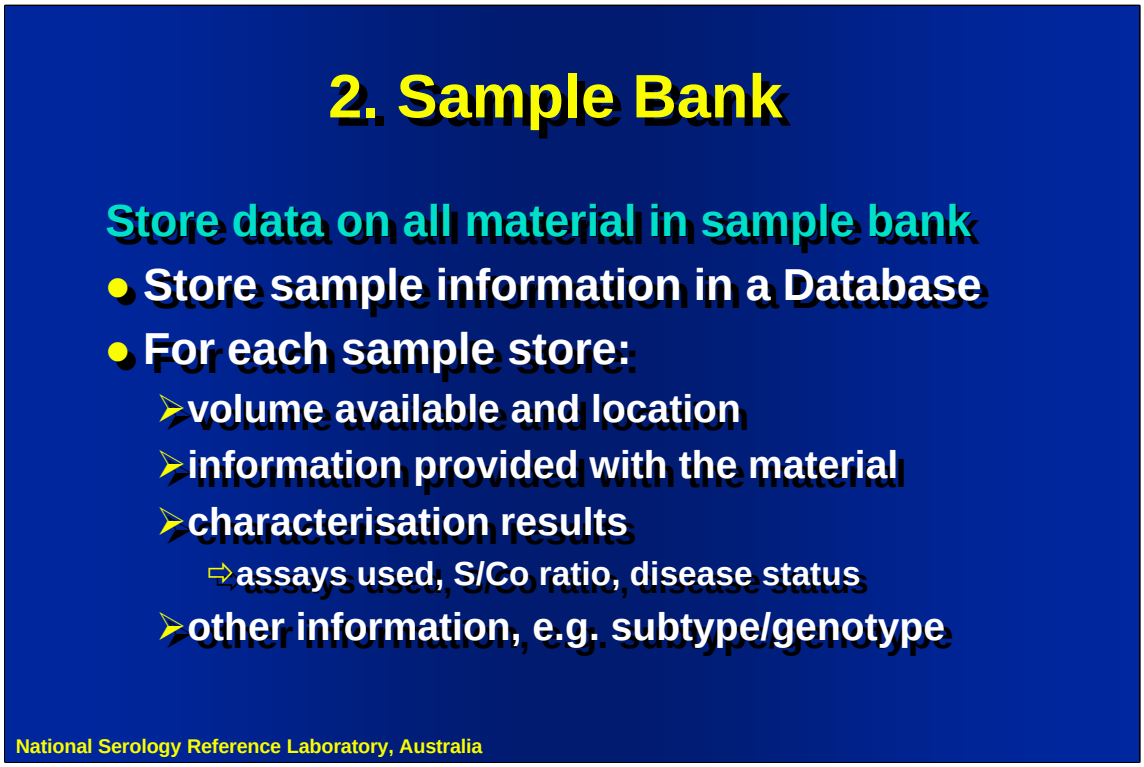
•The samples should be characterised by the provider (or under contract to the provider). A testing strategy should be followed to establish the serostatus of the samples.

•The World Health Organisation (WHO) recommends that two immunoassays and one specific supplemental assay be used to establish the serostatus [WHO, 1996].

•The immunoassays may be of plate, automated or rapid type but the two assays should differ in construction and antigens. The use of two immunoassays is an absolute minimum for characterizing EQAS samples.

•A supplemental assay, such as a Western Blot or LIA, should be used to confirm the serostatus of the specimens ***wherever possible***.

•Ideally all samples should be tested for the presence of hepatitis B and C. In order to minimise the risk for laboratory staff, samples reactive for hepatitis B and C should not be used for an anti-HIV testing panel (unless it is a combined HIV, HBV proficiency panel).



2. Sample Bank

•When storing EQAS samples in a sample bank, it is essential that an accurate database be developed and kept updated. Store as much information as possible about each sample in the database.

•If possible the database should be electronic because this will allow for easy retrieval of information (e.g. data can be stored in Microsoft Excel or Microsoft Access).

This database will help with selection of EQAS panels.

•All information maintained in the database should be linked to the unique identifier of the samples in the bank.

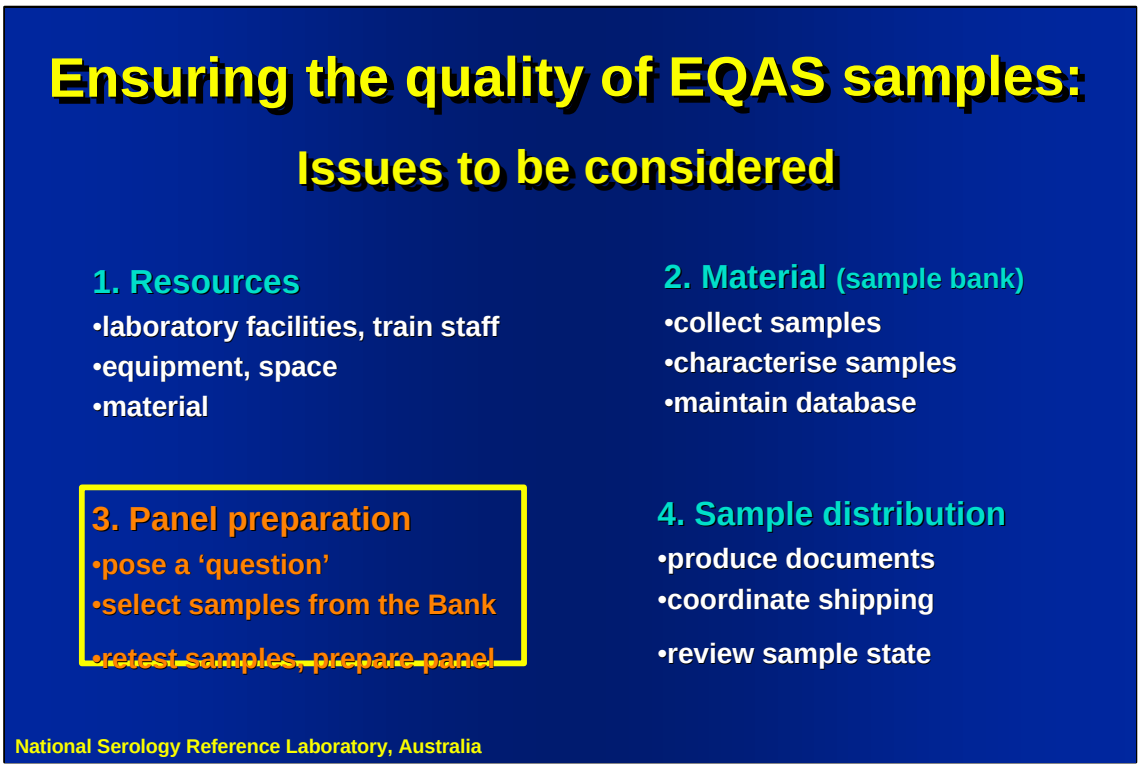
•The database should identify the volume and location of the specimens.

•It is critical that this information is updated each time any volume is removed.

•The database should be used to store all information that was supplied with the samples as well as any test results and the samples HIV status.

This is a picture of a Filing System (or database) in Indonesia

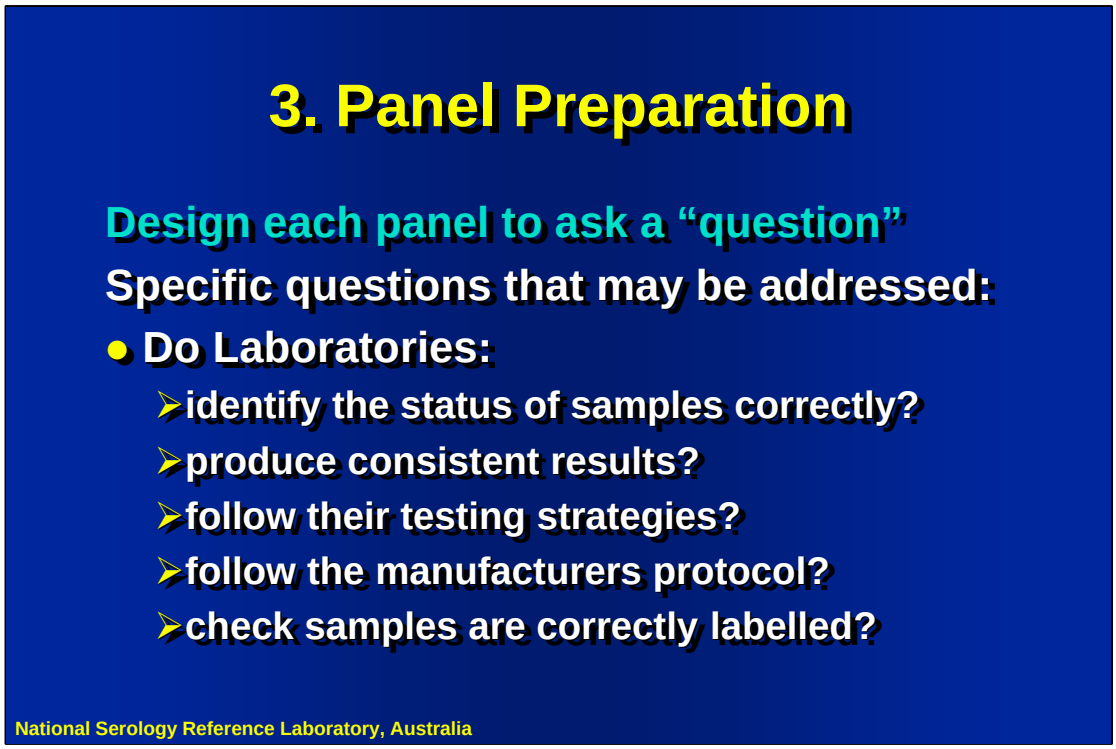
Ideally, a computer database should be used but a system like this can be used if it is done accurately.



Ensuring the quality of EQAS samples:

3. Preparing the panel of samples.

Remember that we need to use designated procedures to ensure the quality of the samples.



3. Panel preparation

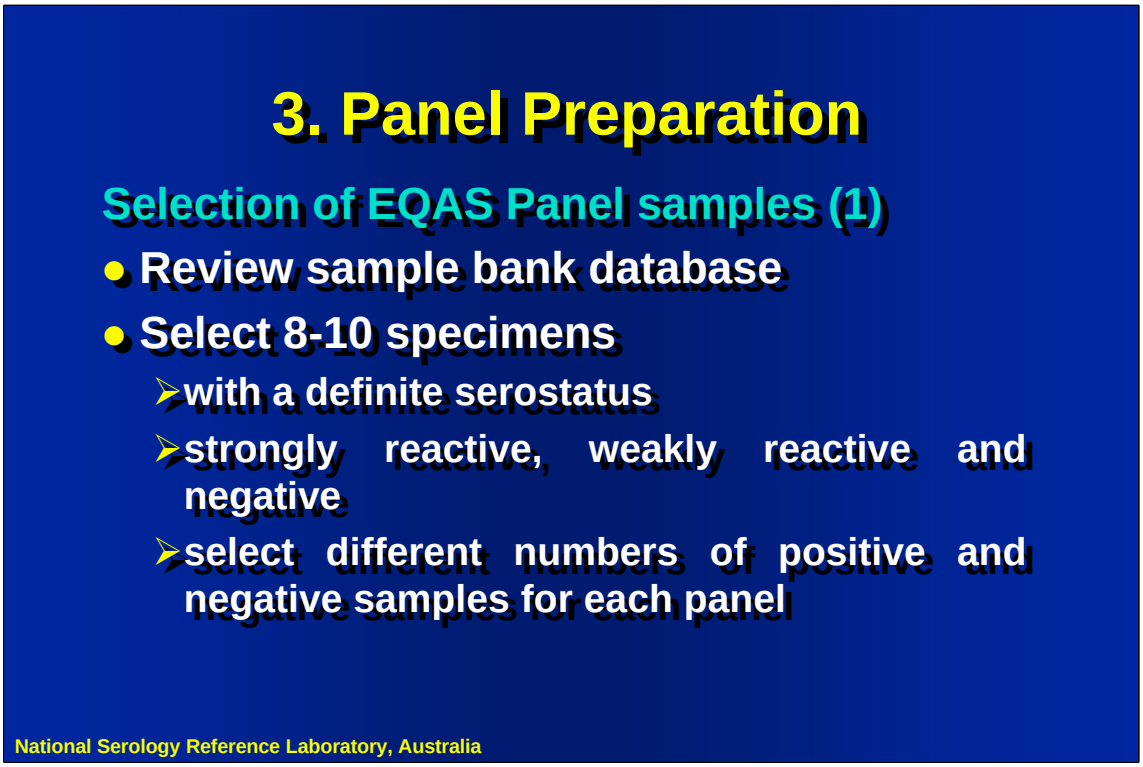
Each panel should be designed to address a specific "question". A "question" is where we assess one specific aspect of the testing process.

The first question may be: can the laboratory correctly identify positive samples as reactive and negative samples as non-reactive?

The questions may become more complex as the scheme progresses.

•For example, the provider may want to address whether a laboratory produces consistent results? The same sample is included several times in one panel (usually close to cut off value ~ S/Co ratio 2-3).

•The EQAS should be an evolving process. As tests, strategies and skills develop, the questions posed in each of the panels should also evolve. Over time, the samples may become more challenging



3. Panel preparation

Once we have a specific "question to assess one specific aspect of the testing process we need to select samples for the EQAS panel.

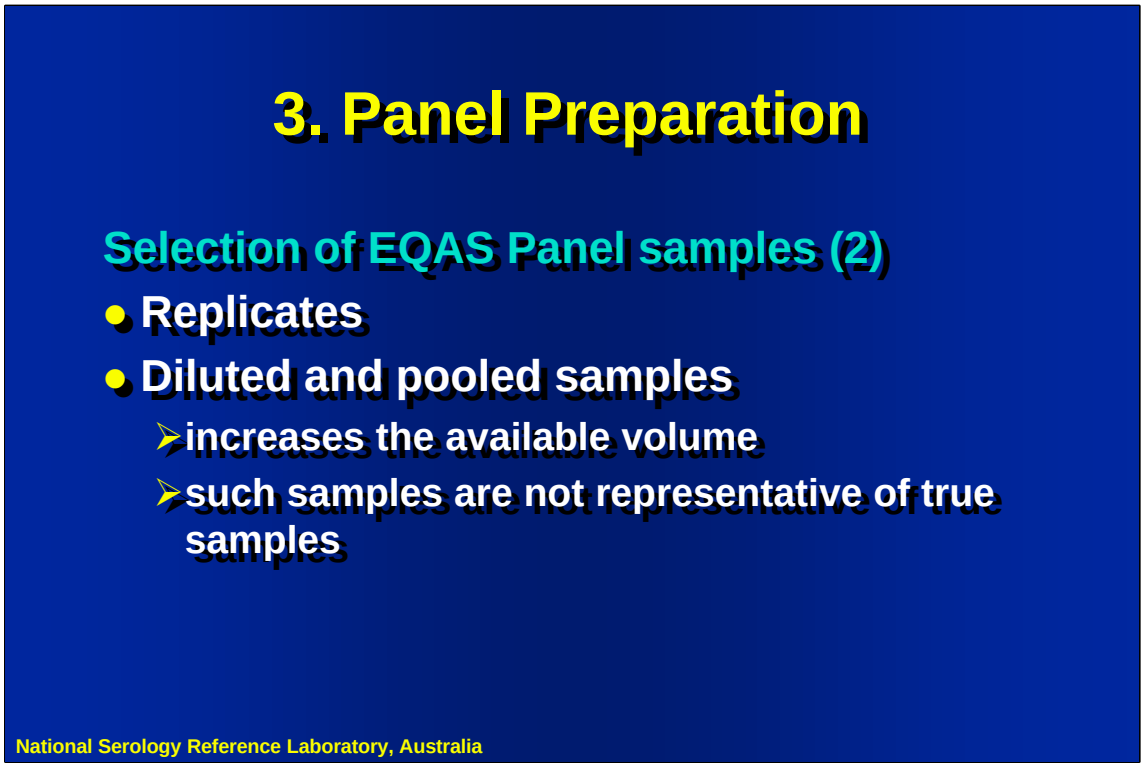
Samples can be selected from our Sample Bank database.

EQAS panels should contain 8 to 10 specimens. A higher number of samples will be more likely to detect errors but too many samples is perceived as burdensome to most EQAS participants.

Each panel should contain good quality samples that have a defined serostatus. EQAS samples should be as similar as possible to real clinical samples. This will ensure that meaningful results are returned by participants.

•Most EQAS providers would include strongly and weakly reactive samples and negative samples in at least early panels and then intermittently. Initially, the panels should not be too challenging but as time goes on the samples can become more challenging.

•The number of positive and negative samples in each panel should not be fixed or predictable.



3. Panel preparation

Selection of EQAS panel specimens (2)

Replicates

When asking your "Question" it is possible to **monitor the reproducibility of the results**. This can be done by including a sample more than once. The replicated samples should be presented as different panel samples with separate identification numbers. Including a sample more than once can also be used to assess the reproducibility of the sample production.

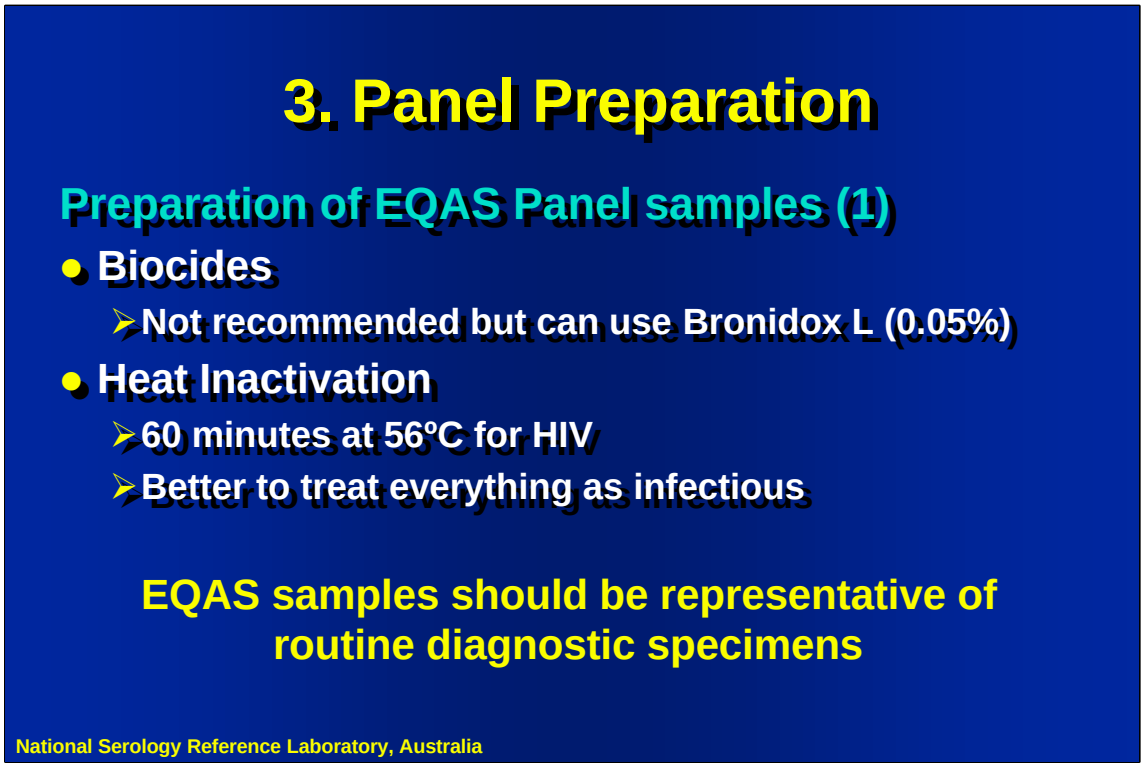
Diluted or pooled samples

•The advantage to using Diluted or pooled samples is that the available volume is increased. However, **samples that have been diluted or pooled are not representative of true samples and will not give consistent results**.

•Unless participating laboratories routinely test pools, it is recommended that panel specimens should not be diluted or pooled because subsets of antibodies that are detected by some test systems, but not by others, will be diluted.

•However, weakly reactive samples are difficult to obtain and **it may be necessary to dilute a strongly positive sample with negative serum at times**. The diluent should be human serum, preferably from a single donor, tested and found to be negative in all available HIV tests. After dilution or pooling, specimens must be well mixed and retested.

•If the provider includes diluted samples in the EQAS panel, the results reported for the samples must be interpreted with caution. Not detecting antibodies in a diluted



3. Panel preparation

EQAS panel samples can be treated to ensure sample integrity.

Biocides

•**Biocides can be added to panel samples to prevent growth of contaminants. However, the use of biocides is not recommended because EQAS samples should be representative of routine samples.** Contamination of EQAS samples is less likely than for quality control samples because there is restricted entry to the tube and storage times should be relatively short.

•If biocides are to be used the provider should perform testing to ensure that the treatment does not adversely affect any assays used in the region of the EQAS.

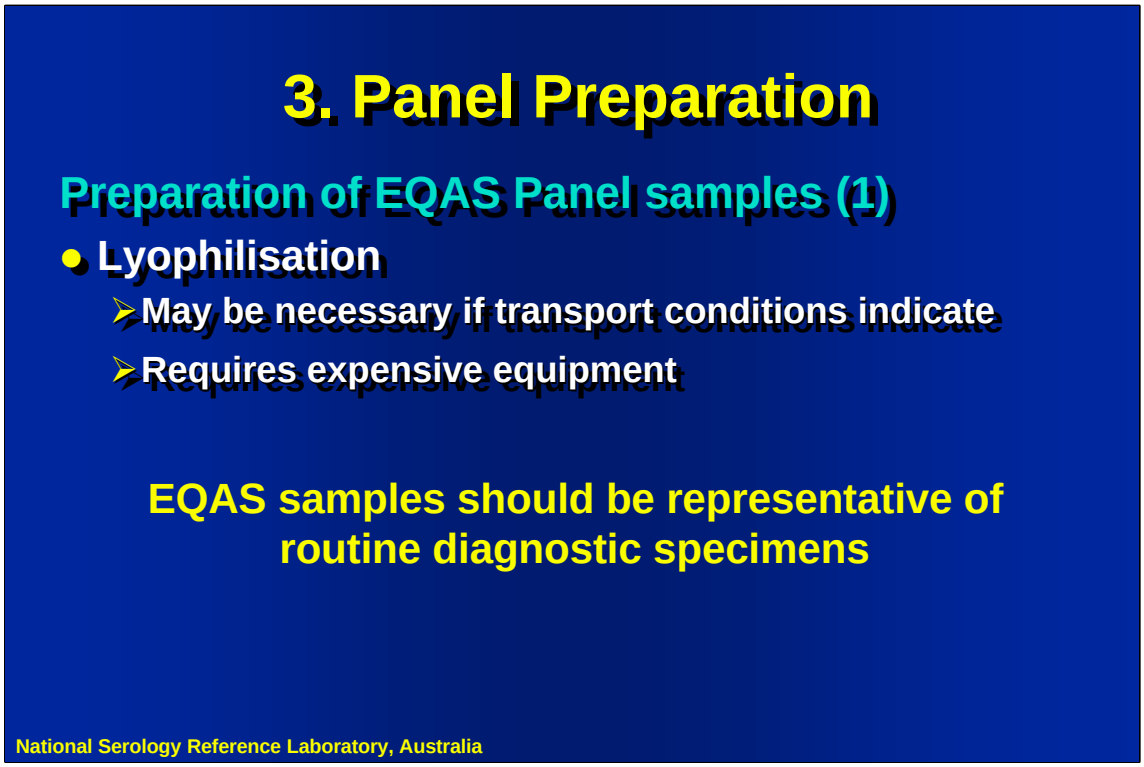
Heat Inactivation can also be performed for safety reasons

•HIV positive specimens may be heat-inactivated (60 minutes at 56ºC) if necessary.

•HIV negative specimens should not be heat-inactivated as this may cause false positive reactions.

•**Heat-inactivation is ineffective against Hepatitis B and C virus**. It is our experience that it is more appropriate to instruct participants in all EQAS programmes to handle samples as potentially infectious and to use the appropriate precautions.

•**EQAS samples should be representative of routine samples**



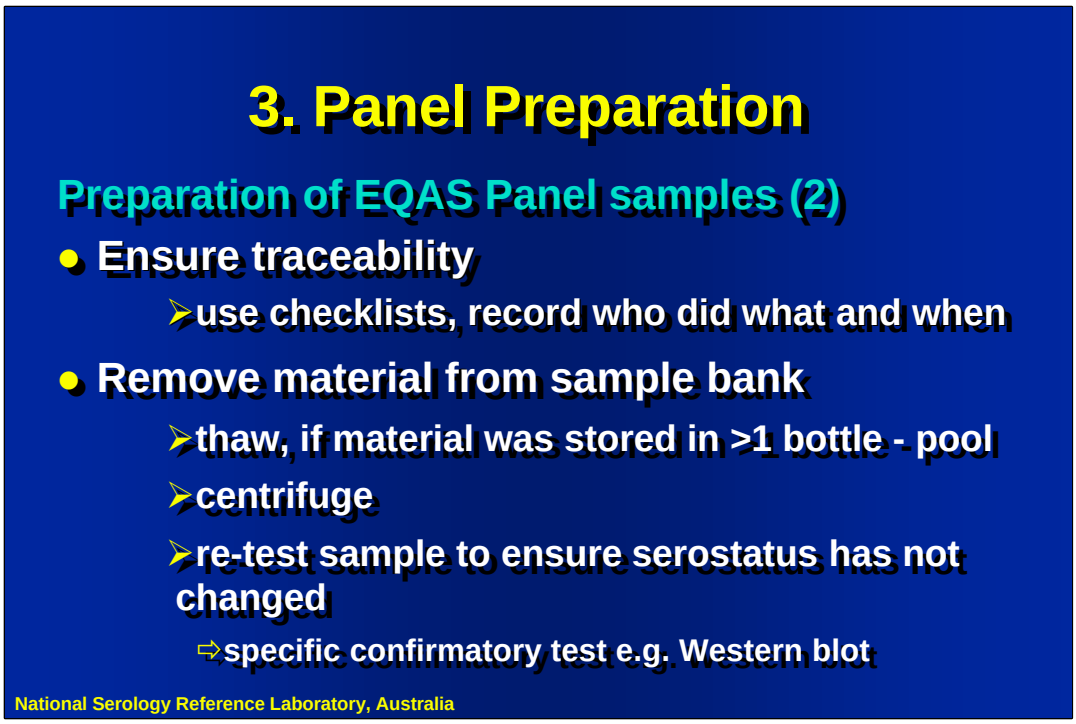
3. Panel preparation

Preparation of EQAS panel samples (1)

Lyophilised samples

- Under very harsh or extended transport conditions, lyophilised samples may be considered.
- However, the participants must reconstitute each sample correctly for the results to be consistent.
- Furthermore, the equipment to lyophilise large volumes of material can be expensive and beyond the means of the provider.
- Lyophilised samples are not representative of routine samples and their activities may be altered.

- **EQAS samples should be representative of routine samples**



3. Panel preparation

When preparing EQAS panel samples it is a good idea to use a dedicated checklist to ensure the quality of the samples and that all steps are accomplished. A checklist can also be used to trace back to find the staff responsible for any errors. These staff can then be retrained if necessary.

The samples will need to be removed from the sample bank and thawed.

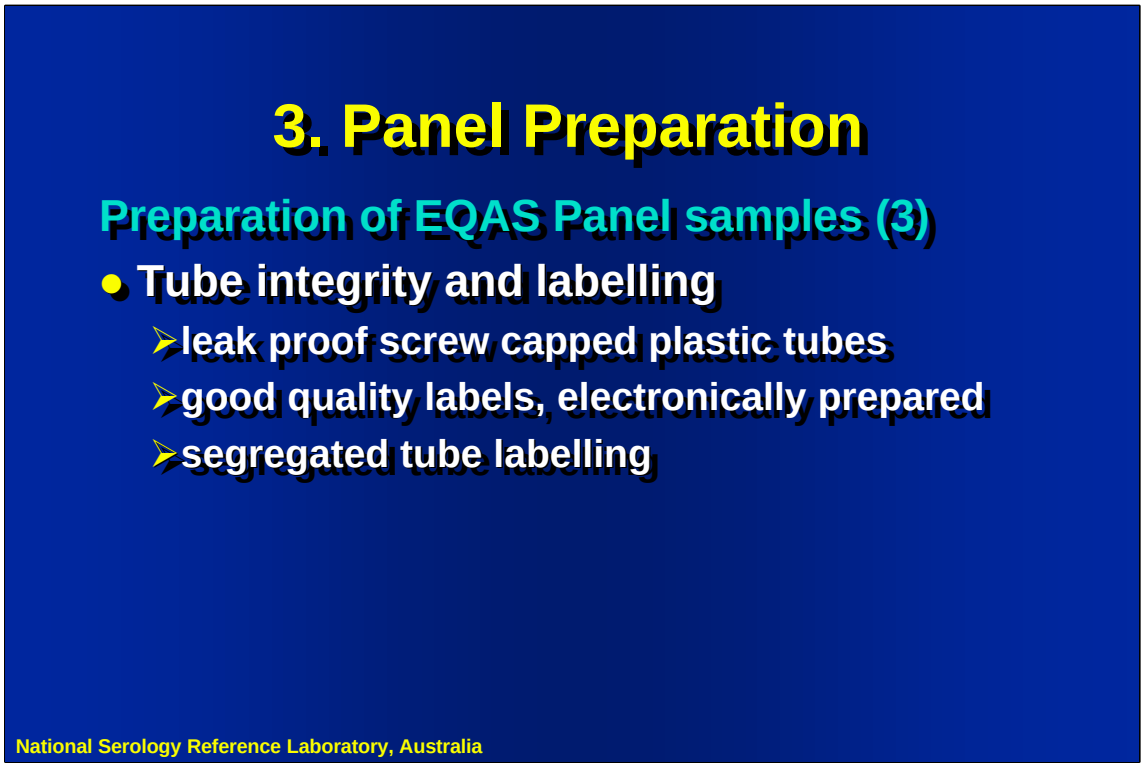
•If the stock material were stored in more than one bottle, the stock material should be thawed and then pooled into one container.

•Stock material should be centrifuged to remove possible clots and spin down any sediment.

•The samples should be re-tested to ensure that the serostatus has not changed during storage. Easiest to re-test on the specific confirmatory assay.

•In all cases, **the most recent status should be taken as the status** result and incorporated into the reports.

•Update the sample bank database - new volumes and test results.



3. Panel preparation

If the re-test results for the stock material are appropriate for the EQAS samples, the material can be **aliquotted into pre-labelled tubes**. It is very important that the tube labelling and alliquotting is of a high standard as many mistakes can be made during this part of the process.

Labelling

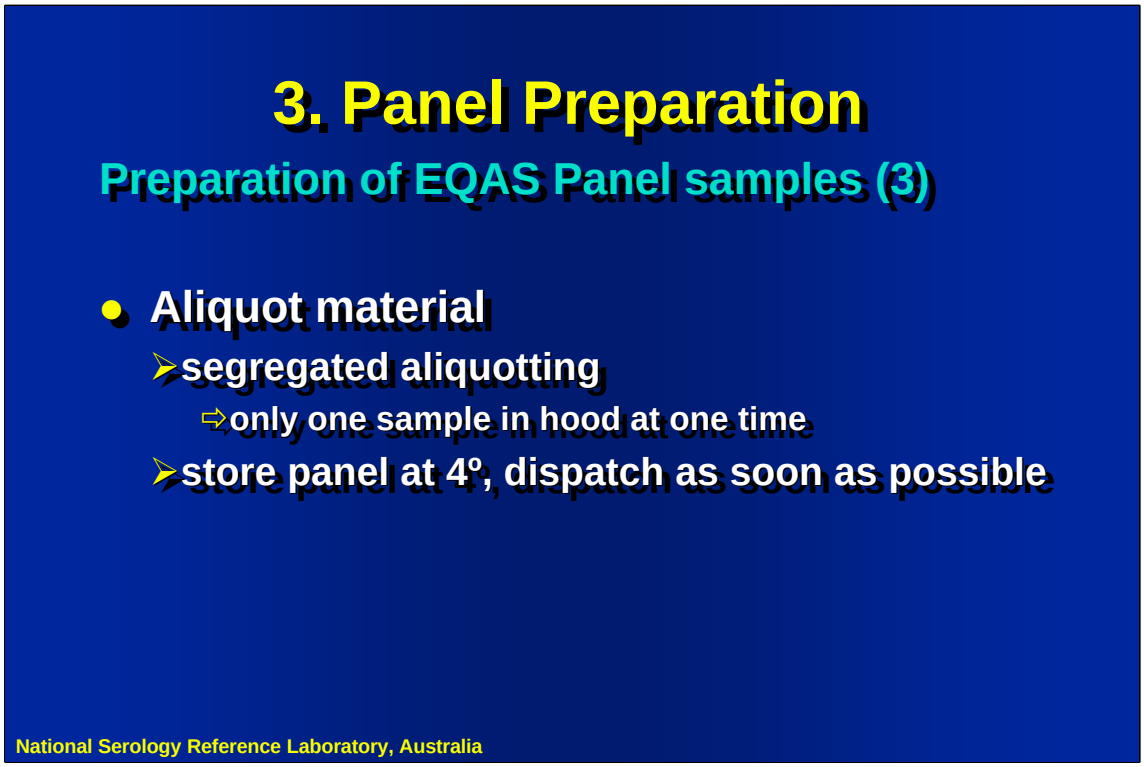
•Tubes should be properly labelled with a number to identify the distribution and with a consecutive number for each specimen

•Ideally the **labels should be generated electronically** using a computer and printer so that labelling errors are minimised.

•To avoid mix-ups during labelling, the samples and their respective labels should be kept apart to minimise the risk of labelling tubes with the wrong labels.

•For example, when labelling Sample 1, the Sample 1 tubes and labels should be physically separated from the Sample 2 tubes and labels.

•All labelled tubes should be checked prior to aliquotting to ensure that the correct sample is put into the correct tubes.



3. Panel preparation

Once the tubes are labelled the samples can be aliquotted into the appropriate tubes.

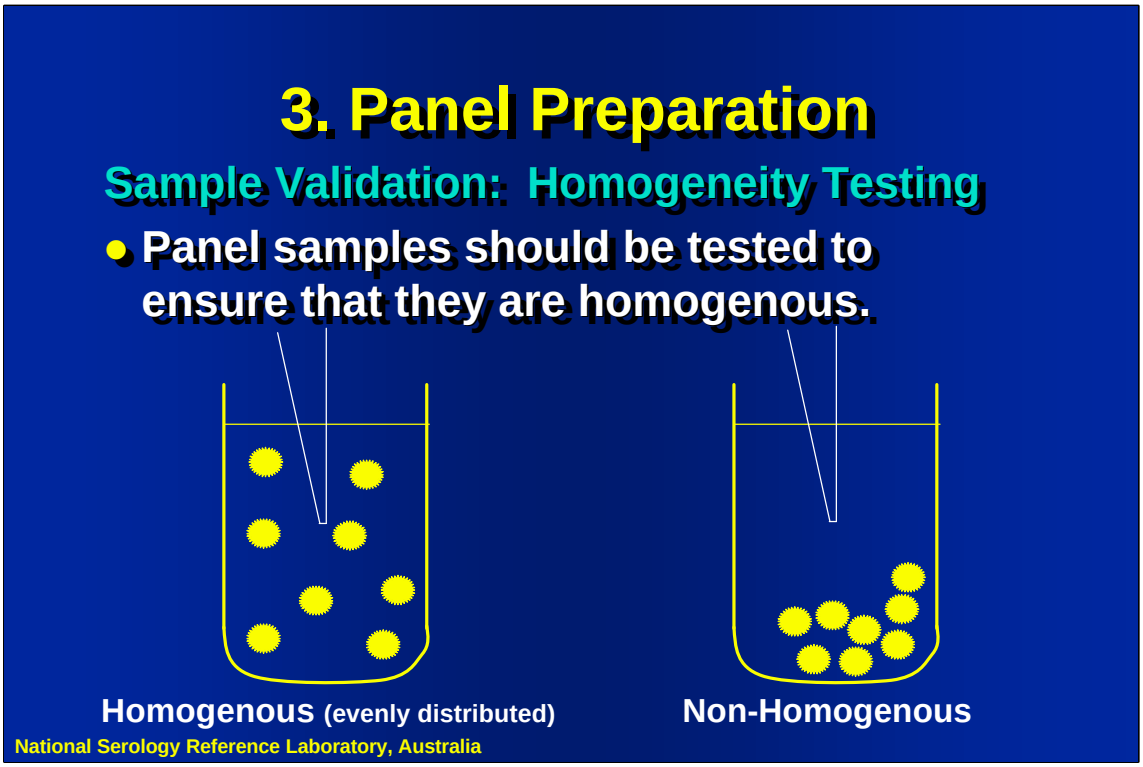
•**Segregated aliquotting** -- we perform segregated aliquotting to reduce the risk of contamination between samples.

•In our experience **we only deal with a single sample at a time**. When aliquotting Sample 1, only Sample 1 labelled tubes and Sample 1 stock material are allowed in the cabinet.

•Once the panel samples are aliquotted, the panels must be **stored at +4ºC** or less **prior to shipping**. Samples should be dispatched as soon as possible.

•Use a checklist and have the staff authorise it when the panel production is complete. This will ensure that all steps in sample production are completed.

•It is a good idea to produce **extra panels** in case a laboratory should not receive the panel in good condition or not at all. Then a second panel can be distributed to that laboratory if requested.



3. Panel preparation

When producing EQAS samples it is important that the samples are homogenous.

i.e. each aliquotted tube of the same sample should contain the same amount of antibody. So that under the same conditions, every sample should produce the same result.

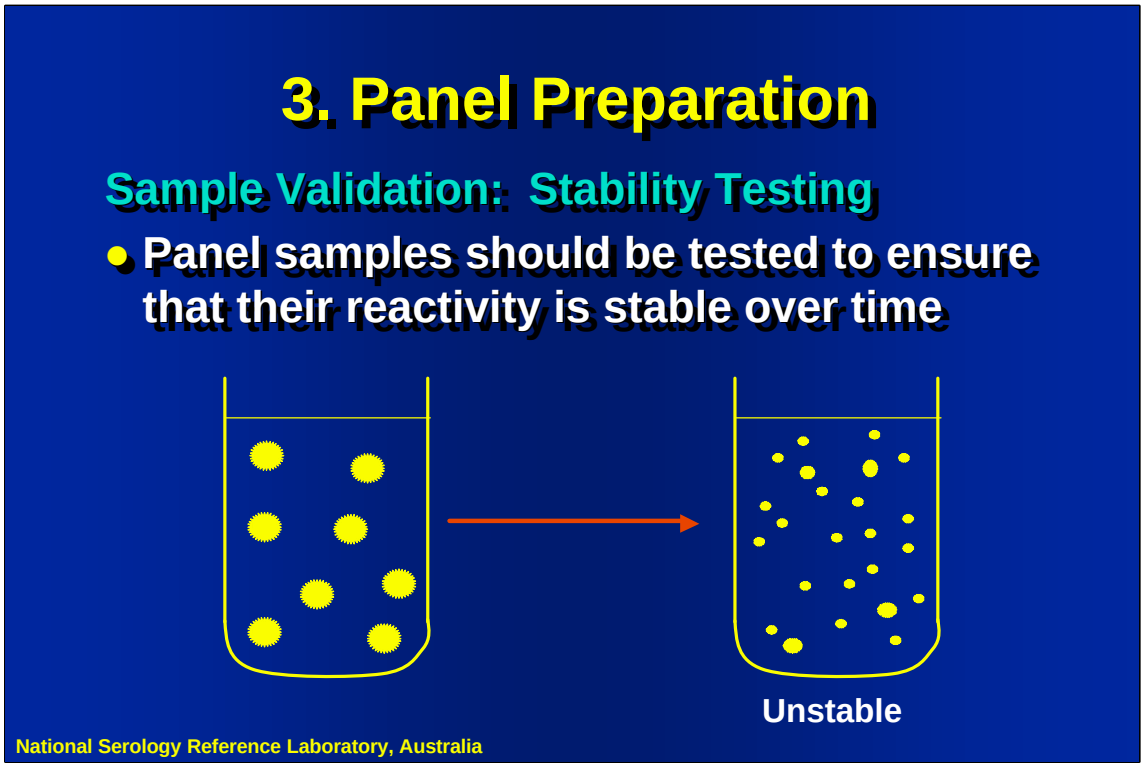
To ensure that samples are homogenous, the **stock material should be thoroughly mixed** prior to testing and aliquotting. Larger volumes should be mixed overnight at 4ºC with a magnetic stirrer.

Homogeneity testing should be performed on a selected number of samples. Make sure that the reactivity of these samples is similar.

Example of a protocol for producing homogeneous samples for an EQAS:

•Test approximately ten aliquots of a sample on a single test run (preferably on an EIA that provides a numeric output).

If only a rapid assay is available then ensure that the reactivity is unchanged for <u>all</u> aliquots. Checking the colour of the spot by eye can give a fairly reliable estimate of variability.



In addition to being homogenous, good quality samples will have a reactivity that is stable over the course of the scheme.

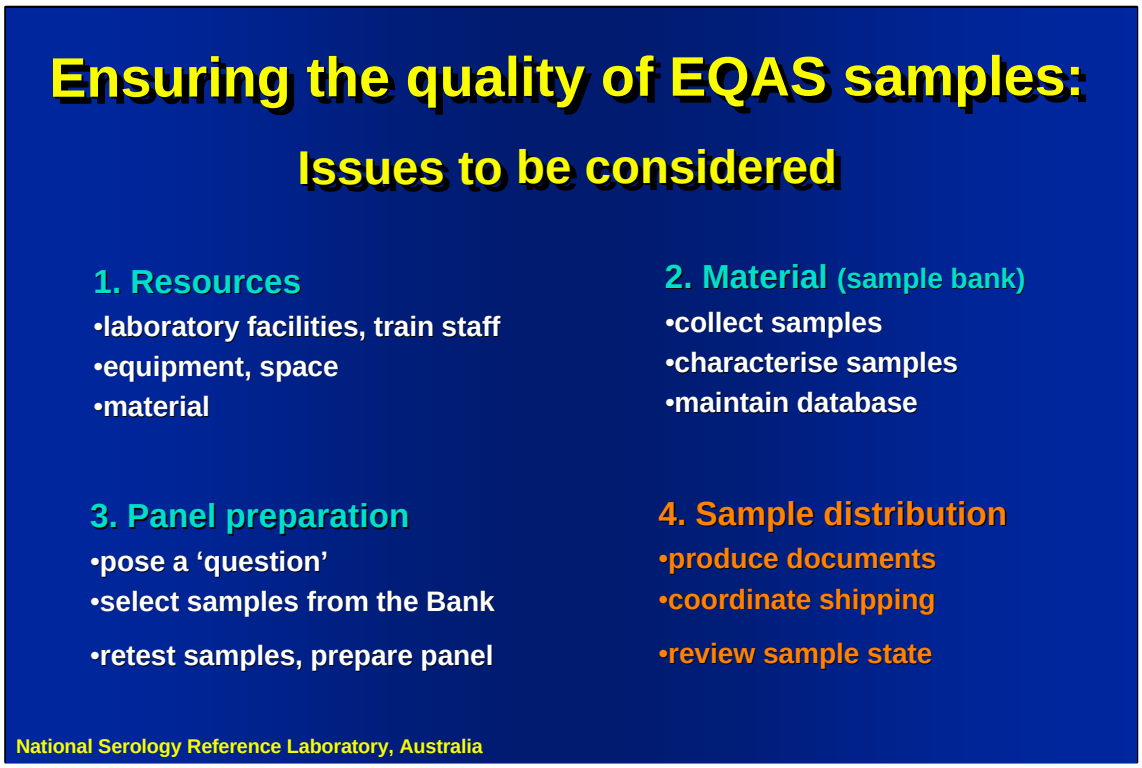
The samples must be stable throughout their time in storage and through all transport conditions to which the panel may be subjected.

•The stability of the sample should be assessed to gauge the rate of possible deterioration. The stability requirements depend on the expected storage and transport conditions of the panel. For example, if a panel is to be shipped to a region, it may be at ambient temperature for a few weeks and may be exposed to extreme temperatures. It is critical that the panel pass stability testing at the expected conditions.

An example of a stability testing protocol follows:

•A participating laboratory whose delivery is expected to take the longest, should be sent and receive two complete panels. This laboratory is requested to return one panel, unopened, to the provider. The provider then tests this panel to verify that the specimens have remained stable, i.e. the test results are the same as before shipment.

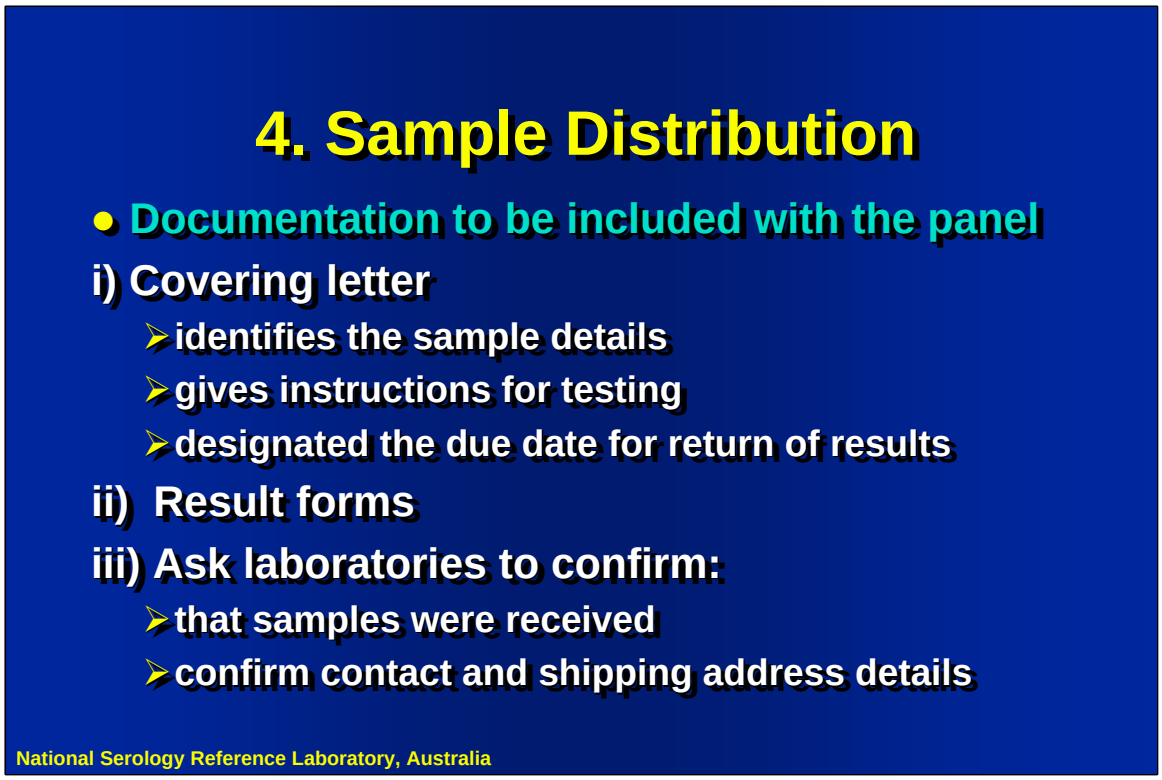
•**If specimens were found to have deteriorated during transportation then alternative distribution channels or lyophilisation might be considered for subsequent panels.** However, the provider would need to demonstrate both the stability of the lyophilised specimens and their unaltered behaviour in any of the test systems [Middle, J.G. *et. al*., 1998].



Step 4 for ensuring the quality of EQAS samples:

4. Distribute panels for EQAS ensuring transport requirements and regulations are followed.

Remember that the samples are out of your care during shipping and this is when the samples are at their most vulnerable.



4. Sample distribution

An EQAS provider should prepare documentation to be included with the panel of samples

<u>Cover letter or instruction sheet should contain</u>:

•**How many samples, Type of samples, Details of how the samples are labelled**. This will allow participating laboratories to check that they received the correct samples.

•The cover letter should advise laboratories to **test the panel as they would a normal diagnostic sample**. The participating laboratories should be reminded that they will gain greater benefit from an EQAS if they are to treat the specimens in the manner that samples would be tested routinely.

•a statement about **potential risk of infection**, even if samples were heat inactivated. The use of universal precautions should be recommended.

•**a deadline** for when results will be considered as part of the analysis and report. Results received after the deadline should not be accepted for analysis or reporting.

<u>Results form</u>

•**Participants should record their results on a specially designed report form**.

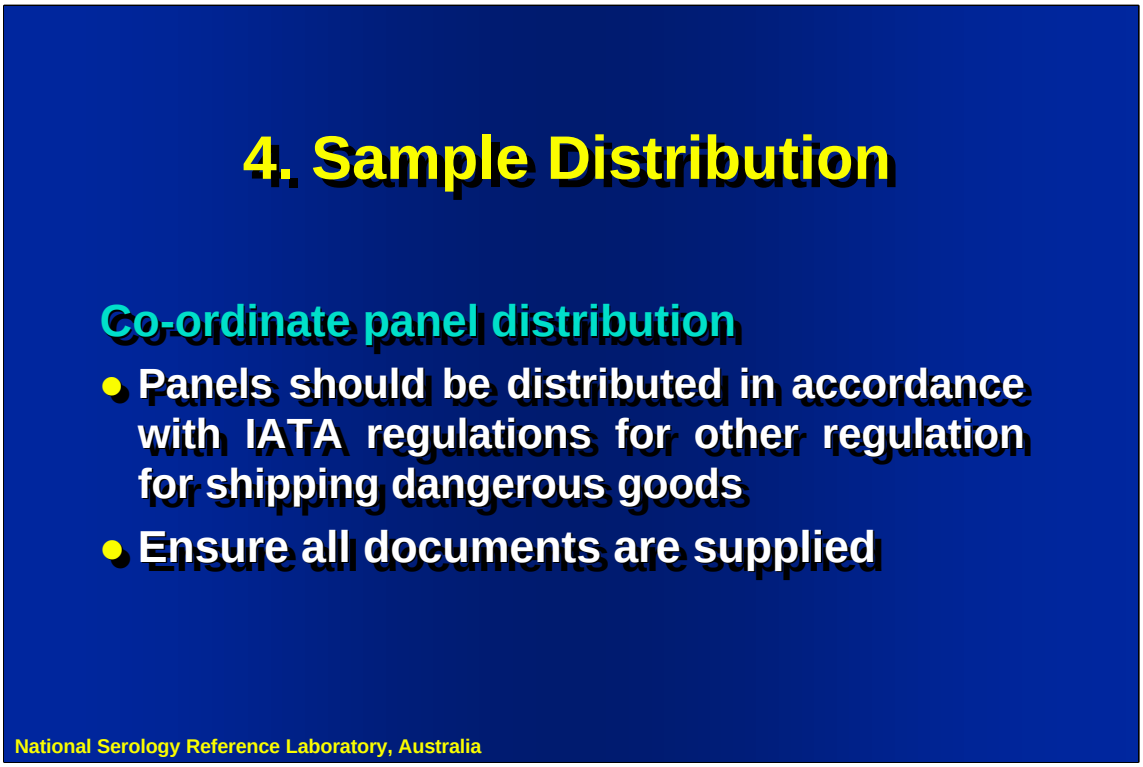
•The distribution number of the EQAS panel as well as identification for each participating laboratory should be clearly indicated on this document.

<u>Reply form</u>

Include a form with the samples which should be sent back by participants as soon as possible to **indicate:**

•**that the panel was received**

•that the shipping address was correct.



4. Sample Distribution

Once the paerwork is done the EQAS samples should be distributed in a safe and cost-effective manner. The quality of the samples must be maintained during shipment.

•Ship according to national and international regulations. Ensure that you have a shipping officer certified as competent in carrying out regulations (if applicable). Remember that you are shipping HIV positive samples that could potentially infect someone, so the samples should be packaged very well.

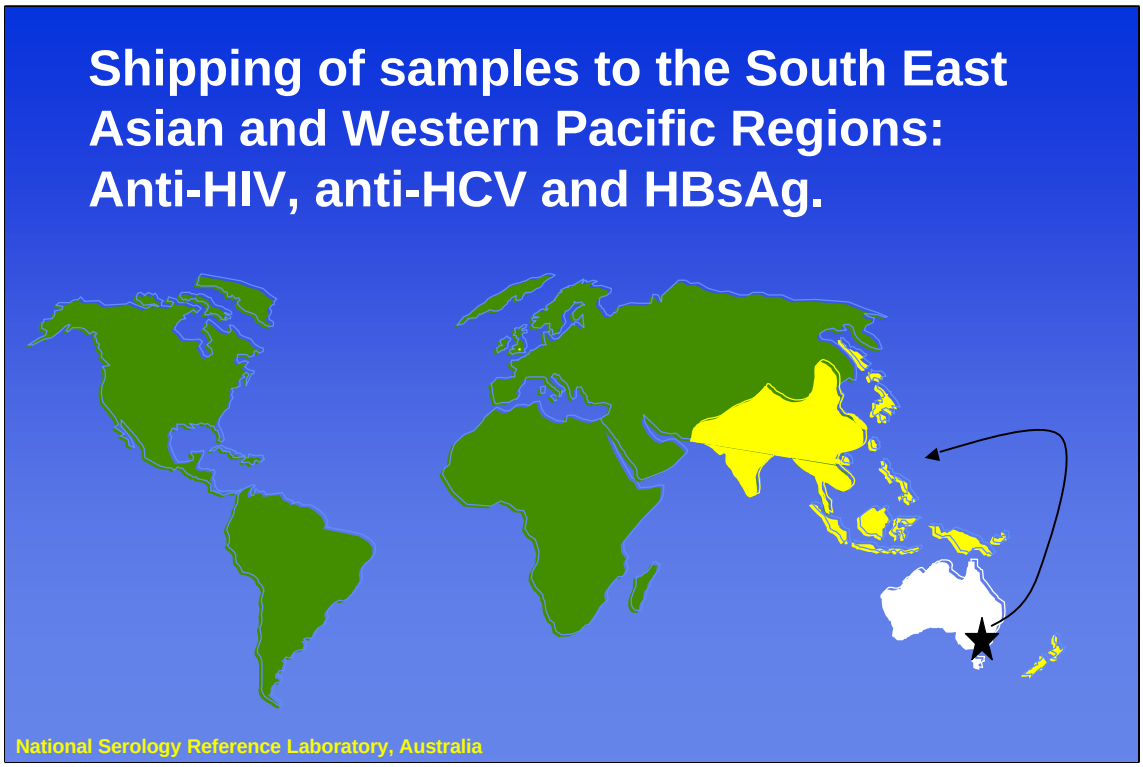
•Ensure that the relevant documents and import permits are supplied with the package.

•Often the postal service is the only realistic means of transmitting EQAS specimens, although **a courier service** or other communication network **should be used where possible**. In some settings, delivery by the provider may be the only solution.

•The specimens must be packaged to avoid leakage.

•Shipping at ambient temperatures should be adequate if stability of the samples has been confirmed. This method is the most cost effective.

•The sample integrity is probably the most important element in the EQAS process. If the sample integrity and quality is common to all participants, results can be compared.



Since 1989 the NRL has been providing an anti-HIV EQAS to the South-east Asian and Western Pacific **regions**. Currently we have 68 participants from 28 countries.

The NRL experience

Problems encountered

<u>The main problems encountered with shipping:</u>

•It is difficult to get accurate contact details because the contact person often changes or the contact person asks for the samples to be sent to different addresses and/or a different person.

-A simple contact **database is ineffective** because of the **complex nature of the shipment**, e.g. some participants receive the shipment via another group in their own country (see below). This means that some **paperwork has to be done manually**.

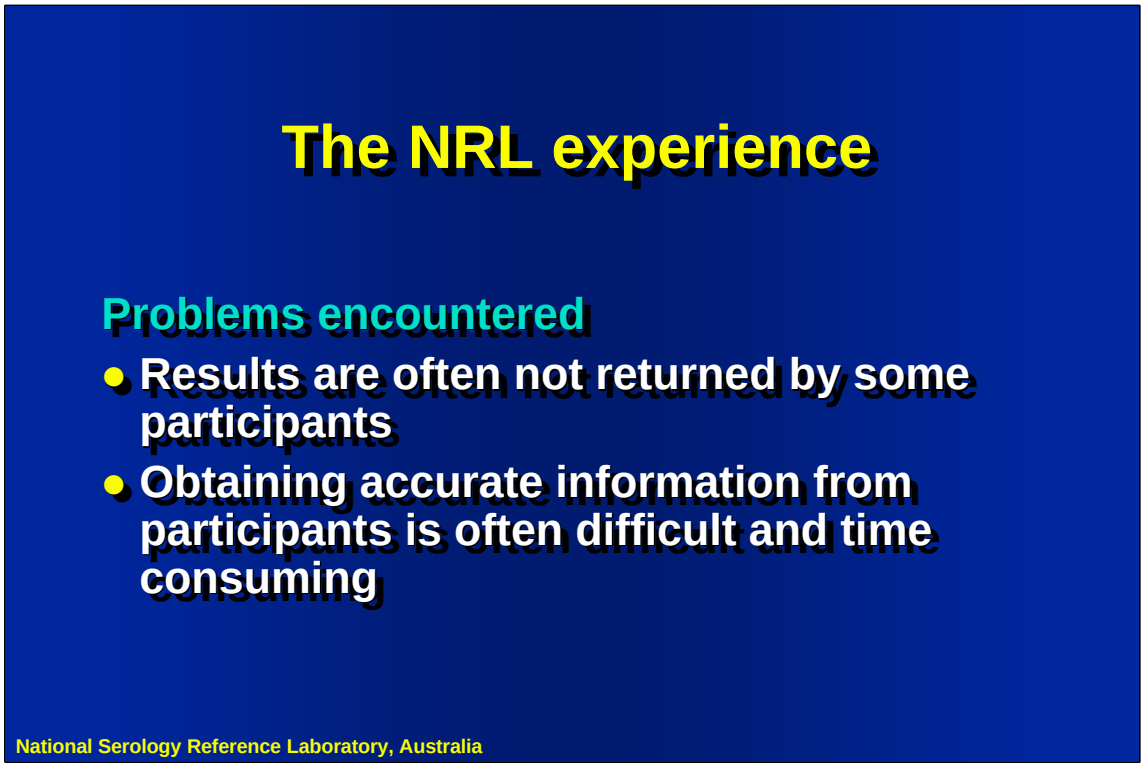
-Some countries have their own customs declaration letter or customs regulations which necessitates further complications.

The import permit is often cheap and easy to obtain but participants may be required to pay for “extra expenses”, sometimes out of their own pockets.

Communication with participants

•**Language barrier** -- **misunderstandings can occur**.

•Lack of a fax or e-mail in some laboratories can make date return and communication very slow because we have to rely on surface mail. Participants who do not respond to our telephone calls, fax, letters or emails must be contacted in person by a collaborator in the country in question.



The NRL experience

Often results are not returned by participants

Common reasons that results are not returned by participants are:-

-"Couldn't get the import permit to clear dangerous goods through customs"

-Shortage of reagents so can't perform the testing

-"Samples were left sitting in the cold room because a new staff member did not know anything about it"

Often the NRL cannot contact participants at all.

Obtaining accurate information from participants is often difficult and time consuming

Problems with analysis of the data

Obtaining **accurate information about an assay** used can be very difficult. Many assays that are not used in Australia are used in the SEA/WP regions. It is **important to try and verify which assay was used** so that results from different assays are not **grouped together for analysis**.

Clear instructions on the results entry form can help improve the accuracy of the information returned to the EQAS provider.

We are very lucky in that we have Thein Thein who spends many many hours chasing up participants to clarify what assay they are using and so on.

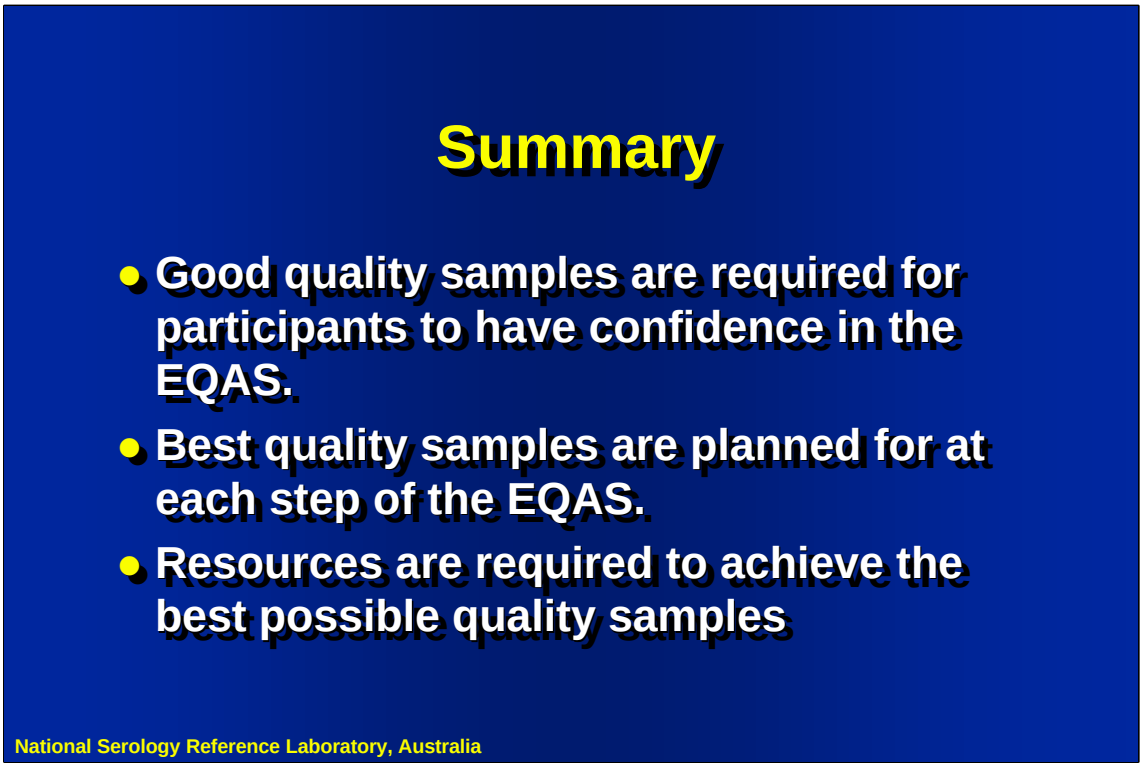
In fact she probably spends up to half her time co-ordinating our EQAS for the SEA/WP regions.

I just wanted to point out that a considerable amount of time is spent running some of these programmes and some staff are dedicated to it almost full time.

The NRL experience

Tips for minimizing shipping costs

- **Select a good courier company** that can safely transport infectious substances effectively.
- **Investigate sending one large package, containing more than one panel** of EQAS samples, to a collaborator who then distributes the panels within their country or region.
- The NRL has 5 contacts that re-distribute panels and this saves ~US$2,400 per panel distribution. This also prevents participants from dropping out of the EQAS because of difficulties encountered in the customs procedures.



Summary

Good quality samples are required for participants to have confidence in the integrity and usefulness of the EQAS.

Participants must be confident that any poor results are due to a problem in their laboratory and are attributable to poor quality samples.

Much of the time and effort involved in providing an EQAS should be devoted to achieving the best possible quality samples.

References

Libeer, J.C. *et al*. Characterisation and classification of external quality assessment schemes (EQA) according to objectives such as evaluation of method and participant bias and standard deviation. *Eur J Clin Chem Biochem* 1996; 34:665-678.

Middle, J.G., Libeer, J.-C., Malakhov, V and Penttila, I. Characterisation and Evaluation of External Quality Assessment Scheme Serum. *Clin Chem Laboratory Med* 1998; 36(2):119-130.

WHO. Guidelines for organizing national external quality assessment schemes for HIV serological testing. January 1996.


National Serology Reference Laboratory, Australia