



QCONNECT LIMITS

Investigation of Unexpected Quality Control Results

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QConnect Limits:

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Background: The use of a Quality Control (QC) sample independent of the controls provided by the test kit manufacturer (kit controls) is mandatory in some jurisdictions and highly recommended by others. For infectious disease serology testing, a QC sample having low positive reactivity should be tested periodically, usually daily or with every test run, and the results monitored by plotting on a Levey-Jenning chart. Those responsible for monitoring the QC results are required to establish a range within which the results of the QC sample are expected to fall. Traditionally, the upper and lower values (control limit) are established by testing multiple replicates of the QC sample over a period of time (often cited as 20 times) and calculating the mean (\bar{x}) plus and minus two or three standard deviations (SD). When subsequent results of the QC sample are outside the control limits, an investigation is triggered.

These traditional methods for monitoring QC results have deficiencies when applied to infectious disease serology. Control limits established using a relatively small number of QC results may be too stringent. When a new lot of reagent is introduced, frequently the reactivity of the QC sample falls outside the control limits, even when the kit control results are within the manufacturers' validation criteria. Those responsible for releasing patients' results are faced with a dilemma. Do they ignore the external QC results and release the patient results based on the kit controls acceptance criteria or do they reject the run and institute an investigation into the cause of the change of reactivity of the QC sample?

A QC program for infectious disease serology, called QConnect, has been conducted by NRL for over a decade. Samples with low-level reactivity have been identified for use in specific serological test kits (assays) and the results of testing these QC samples have been entered into NRL's QC monitoring software, EDCNet. Data have been extracted from EDCNet and analysed to establish control limits for Assay/QC combinations (peer groups). For example, users of the Abbott PRISM HCV ChLIA have tested different lots of the sample QC sample for over 10 years, with over 100,000 results having been entered into EDCNet. Using these data, NRL has established QConnect control limits for this peer group. The QConnect control limits developed by NRL include all sources of variation expected in a test system, including changes in reagent lot numbers, multiple operators, instruments and instrument maintenance and calibration events. Therefore, results falling outside the NRL control limits are highly unexpected and should trigger an investigation.

Manufacturers of IVDs optimise and evaluate the performance characteristics of their test kit prior to making them being available to customers. Those IVDs that have been registered for sale in Europe, Australia, North America and other developed countries must conform to quality specifications set down for each jurisdiction, which include providing proof of the test kit's sensitivity and specificity when used according to the manufacturer's instructions. All IVDs have Instructions for Use (IFU) which specify its intended use, the acceptance criteria for the kit controls, the method of establishing the test kit's cut-off and how the test results obtained should be interpreted. When a test kit is used according to the manufacturer's IFU, patient results from all valid test runs (runs that meet the manufacturer's validity criteria) should be reportable.

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Sources of variation: Before discussing how to investigate unexpected QC results, a review of sources of variation is useful. The purpose of testing a QC sample is to monitor the variation in results over time. Understanding the sources of variation is useful when troubleshooting unexpected QC test results.

The main sources of variation can be categorised as:

- **Reagent lots** – Often, when a new lot of reagent is released to the market, the QC sample test results may vary from previous reagent lots. Sometimes reagents are manufactured with new raw materials. This is often the case when a new master lot is released. Although the manufacturer will re-calibrate the system to match the new raw material and conduct testing to confirm the sensitivity and specificity of the test kit, a change in QC reactivity is commonly seen.
- **Instruments and equipment** – All instruments or equipment, such as pipettes or plate washers, used to test patient samples can introduce variation into the test system. The volume of reagent dispensed, the temperature and length of incubation, the amount and strength of washing of the solid phase and the reading of the signal are all sources of variation, even in well maintained instruments and equipment.
- **Calibrations and maintenance** – Each time an instrument or equipment is calibrated or preventative or corrective maintenance is performed, the results of the QC may vary. Logging these events is extremely helpful when troubleshooting unexpected QC results.
- **Operators** – Standard operating procedures (SOP) are established by the participant and the IFU test kit manufacturer. However, occasionally these instructions are ambiguous or not followed. For example, failing to allow the reagents to warm to room temperature may result in decreased reactivity or patient or QC samples.
- **Storage and transport** – Most test kit manufacturers comply with strict test kit lot release criteria to ensure the stability of their test kit's performance characteristics. For example, in the EU, each new lot of reagent has batch release testing performed. However, once the reagents are shipped from the warehouse, there is a possibility that the reagents are subject to adverse transport and /or storage environments, which could adversely affect the performance of the test kit.
- **Environmental conditions** – In most developed countries, the laboratory environment is well controlled. Regulated temperature and humidity, access to stable electricity and clean water supply is often taken for granted. This is not necessarily the case around the world, and in certain situations, inadequate environmental conditions can occur, even in developed countries.

QC monitoring is expected to detect a change in reactivity in the QC sample results when a change in the variables described above, is experienced. Although the testing of QC samples cannot be guaranteed to detect all adverse events, when it does, access to information about each of these variables can aid troubleshooting.

Use of QConnect Limits:

NRL provides QConnect limits and a protocol for investigating unexpected QC test results for laboratories seeking guidance. They are not prescriptive and can be implemented in isolation or in conjunction with individual laboratory procedures. Those responsible should determine what method of QC review is appropriate, based on the test kits being used, the seriousness of the disease state under investigations, the risks associated with reporting incorrect patient

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results and any other mitigating factors such as the use of confirmatory test kits and/or procedures to verify test results.

Investigation into unexpected QC test results:

NRL strongly recommends that the manufacturer's IFU are followed.

When the manufacturer's kit control results are outside the validation criteria specified in the IFU, irrespective of the results of the external QC sample, the patient results must not be reported, and an investigation into the cause of the run failure instituted and the root source of the problem rectified before the patient samples are re-tested.

In the situation where the manufacturer's kit control results are within validation criteria specified in the IFU and the external QC result(s) are within the QConnect limits, the patient test results can be released to the clinician.

When the manufacturer's kit control results are within validation criteria specified in the IFU but the QC test results are outside the QConnect limits, the following investigation is suggested:

1. **Review test results and data entry** – The most common reasons for unexpected QC result is due to data entry errors. Check that:
 - a. the numerical result has been entered correctly into EDCNet, especially the decimal point:
 - b. the result has been entered under the correct test kit or test kit version or under the correct analyte (e.g. HIV combi assays may have separate antibody and antigen analytes available).

If the participant has made a data entry mistake in EDCNet, use the EDCNet "Review" function to modify or delete the test result. If the participant has introduced a new test kit, inform NRL by emailing QConnect@nrlquality.org.au.

2. First line check

If no data entry errors have been made and the result is a true result for an appropriate Assay/QC combination, the First Line Check should be performed when a QConnect QC test result falls outside the QConnect limits for the first time in a series. The First Line Check is designed to review quickly, all obvious sources of variation that may have contributed to the change in reactivity.

Review the test process using the following questions:

- a. Is the QC sample past its expiry, including open vial expiry dating?
- b. Is there any visible change to the QC sample such as cloudiness or visible particulate matter?
- c. Has the QC sample been stored under conditions other than that specified in the QC IFU?

If yes to any of these, then discard the QC material and re-run the QC using a new, in date vial.

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- d. Was the appropriate QC tested?

Check that the QC specified for the relevant test kit was used.

- e. Has a new lot of QC been introduced?

If yes, then check in EDCNet to determine if other laboratories are experiencing the same change in reactivity. The change may be due to different factors in the manufacture of the QC sample. If this is the case, the QConnect limits may need to be reviewed. If a new lot of QConnect QC has been introduced and the reactivity is outside the QConnect limits, contact NRL on QConnect@nrlquality.org.au.

- f. Is the test kit past its expiry dating?

If yes, then discard the reagents, re-run the patient samples and QC samples using an unexpired kit.

- g. Has a new lot of reagent, or a new delivery of an old lot or reagent, been introduced?

If yes, has the new lot been commissioned? Check storage and transport temperature if possible. Check EDCNet to determine if other laboratories using the same lot number are experiencing the same change in reactivity (see below). A decision to accept this lot of reagent for general use must be made. This decision will be based on the risk and consequences of reporting an incorrect patient result and therefore will be dependent upon the analyte being tested for and the purpose of the testing (e.g. diagnostic vs blood donor screening). The decision should be taken in collaboration with the test kit manufacturer. It is recommended that NRL is contacted using QConnect@nrlquality.org.au if additional information is needed. NRL is willing to be involved in communications with the test kit manufacturer and to undertake further investigations as required.

- h. Were the reagents stored and processed correctly? Were the reagent or kit controls left in non-ideal environment? Is the environment within acceptable conditions?

Review the refrigeration temperatures, room temperature and humidity and confirm that they meet specified requirements.

- i. Does the instrument require daily maintenance and start-up to be performed? Does the instrument require re-calibration?

If yes, perform the daily maintenance, start-up procedures and/or re-calibration and re-test the QC sample.

- j. Is instrument/equipment preventative maintenance up-to-date?

Review all instrument maintenance requirements and ensure that they are up-to-date. If there are outstanding maintenance requirements, perform the required procedures and repeat QC and kit controls.

- k. Is the operator trained to perform the test? Did the operator follow the SOP?

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Check that the operator followed normal procedure, especially any critical requirements or activities known to influence the test kit performance.

It is recommended that the First Line Check be undertaken and the results documented. NRL has provided a check list (below) that can be used. If all the checks have been performed and no corrective actions are required, the results of the patient samples can be reported. It is recommended that the test results of the QC sample for this test kit are monitored closely and any further unusual reactivity investigated.

If further information or advice is required, contact NRL on QConnect@nrlquality.org.au.

3. Second Line Check:

If subsequent QConnect QC sample test results fall outside the QConnect limits, further investigations are usually warranted. Sometimes, the source of the unexpected test results will have been identified in the First Line Check e.g. when a new batch of reagent is introduced. The participant may decide to accept the QConnect results outside the QConnect limits when they are associated with this reagent lot number. However, when the source of variation is unknown, EDCNet provides the participants tools to undertake detailed investigations.

Are other peer group members experiencing the same issue?

It is important to know if other QConnect members are experiencing the same issue. By using the “Mean/Scatter” report in EDCNet, the QC test data for an Assay/QC combination over a defined period of time can be reviewed. To learn more about how to use the “Mean/Scatter” report, please refer to the EDCNet Handbook or the EDCNet tutorials found in the “Help and Resources” tab of EDCNet.

Briefly, NRL suggests that the investigation process starts by graphing the data using the Laboratory Scatter report. From the Mean/Scatter report, first select the data required using the “QC Results” function.

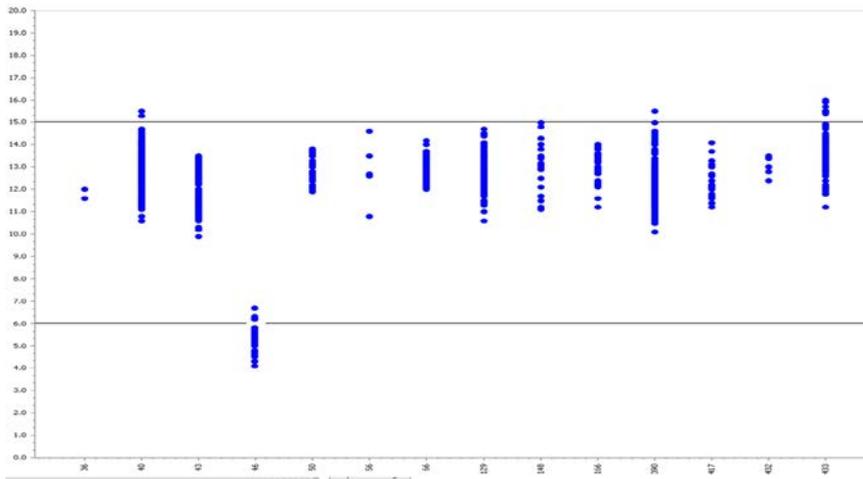
Date Range:	Test Type:	Control:
Custom	Serology	<input type="radio"/> Kit Control <input checked="" type="radio"/> EQC
From:	Test Kit:	Analyte:
01-06-2013	Abbott ARCHITECT Rubella	Rubella IgG
To:	Assay:	EQC Name:
14-07-2014	<input checked="" type="checkbox"/> Select All <input checked="" type="checkbox"/> Abbott ARCHITECT Rubella IgG CMIA	QConnect RubellaG

From the “Mode” function, choose “Graph”, “Scatter” and “Laboratory” as below.

Mode		
View Mode:	Type:	Group:
<input checked="" type="radio"/> Graph <input type="radio"/> Table	<input type="radio"/> Mean <input checked="" type="radio"/> Scatter	<input checked="" type="radio"/> Laboratory <input type="radio"/> Instrument <input type="radio"/> Test Kit Lot Number

The data of all laboratories that have entered data into EDCNet for that Assay/QC combination for the period of time selected will be plotted as below.

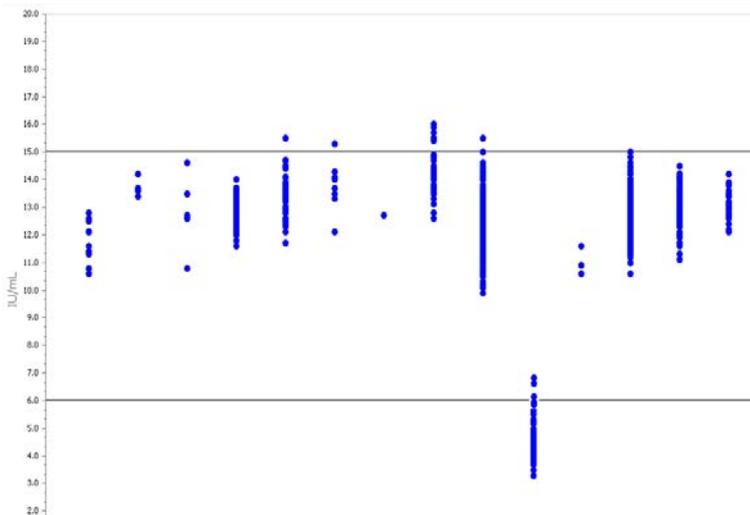
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Compare the position and the spread of results of your laboratory with those of other laboratories.

Laboratory-specific variation:

It is possible that your laboratory is the first to use a new batch of reagent that is performing differently to previous batches. To determine if this is the case -



From the “Mode” function click the “Test Kit Lot Number” radio button. EDCNet will plot the same data points sorted by test kit lot number. Any test kit lot that is producing results that are unusually high or low will be obvious.

By selecting that lot number in the “Filter Mode” and plotting the results as a “Laboratory” report, the laboratories using that particular lot number and their results can be viewed.

Filter

EQC Lot Number:	Test Kit Lot Number:	Laboratory:
<input type="checkbox"/> Select All	<input checked="" type="checkbox"/> Select All	<input checked="" type="checkbox"/> Select All
<input checked="" type="checkbox"/> 330101	<input checked="" type="checkbox"/> 62490UN13	<input checked="" type="checkbox"/> 36
	<input checked="" type="checkbox"/> 03926UN14	<input checked="" type="checkbox"/> 40
	<input checked="" type="checkbox"/> 04814un13	<input checked="" type="checkbox"/> 43
	<input checked="" type="checkbox"/> 09543UN13	<input checked="" type="checkbox"/> 46
	<input checked="" type="checkbox"/> 25247UN13	<input checked="" type="checkbox"/> 50
	<input checked="" type="checkbox"/> 32490UN13	<input checked="" type="checkbox"/> 56
	<input checked="" type="checkbox"/> 39264UN14	<input checked="" type="checkbox"/> 66
	<input checked="" type="checkbox"/> 51869UN13	<input checked="" type="checkbox"/> 129
	<input checked="" type="checkbox"/> 62490UN13	<input checked="" type="checkbox"/> 148
	<input checked="" type="checkbox"/> 88219un13	<input checked="" type="checkbox"/> 166
	<input checked="" type="checkbox"/> 98450UN13	<input checked="" type="checkbox"/> 390
		<input checked="" type="checkbox"/> 417
		<input checked="" type="checkbox"/> 432
		<input checked="" type="checkbox"/> 433

Apply Filters

If the variation is specific to your laboratory, the investigation should focus on the instrumentation/equipment, the processes used by the operator and the transport, storage and use of reagents.

If other laboratories are using the same test kit lot number the investigation should focus on the instrument and processes used. If your laboratory has multiple of the same instrument, the

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results for each instrument can be plotted individually using the “Instrument” option in the “Mode” function. If all instruments are performing similarly, then the variation may be due to the processes used. Review the processes used to obtain the unusual results. This may best be achieved by an independent person familiar with the SOP observing the testing to detect any deviation from the documented procedure. Also investigate how the test kits were handled during transport and storage as adverse conditions may have only affected the test kits delivered to your laboratory. The use of test kits from a previous or new delivery may provide additional information.

It is important to think through each possible source of variation and review EDCNet data to exclude possible causes. NRL suggests that each investigation is documented in a traceable manner, including dates of investigations, copies of graphs and other information stored with signatures of those involved.

Non-laboratory-specific variation:

If all members of the peer group are experiencing similar unexpected changes in QConnect QC results, the most likely source of variation are the reagent or QC lots. Check if a new lot of QC has been introduced? If so, compare the QConnect results of the new lot against the previous lots by using the “Filter” function. If the results of the new QConnect QC are markedly different to that of previous lots of the same QC, contact NRL at QConnect@nrlquality.org.au to investigate.

If the variation is being experienced by some or all of the peer group, determine if a particular test kit lot is the source of this variation by using the process described above. If the unexpected results are found to be due to a particular test kit lot number, notify the manufacturer and NRL for advice.

NRL Investigations:

At any stage of the QC investigation process, NRL would be pleased to offer advice, conduct additional investigations or act as a conduit with the manufacturer and peers. NRL is keen to be involved in investigating unusual QC results. With every investigation, NRL will write and publish a report on the QConnect website. Please feel free to contact NRL for further information or support.



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