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Tips for using wash solutions

Many IDEXX ELISA test kits require the use of a diluted wash buffer between incubation steps. To maintain a high level of ELISA test kit performance, keep these guidelines in mind when using wash solutions:

- Use high-quality distilled or deionized water to mix with the specified volume of a wash buffer concentrate.
- Consult the test kit's insert to check the amount of wash buffer required to run each plate. The wash volume needed may vary depending upon the washer. Automated washers tend to use more wash buffer than manual washers because they need to be primed and then flushed after use.

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Testing the calibration of a microplate reader

It is good laboratory practice to periodically test the calibration of your microplate reader. IDEXX checks the calibration of its readers using calibration test plates, which can be purchased from most reader manufacturers or from laboratory equipment providers.

Important: The calibration plate must be calibrated for the wavelength you normally use for testing (e.g., 630 nm, 650 nm, 450 nm). The calibration can be performed by the manufacturer of the calibration plate.

Use the following steps to run a calibration test plate and to print the results:

1. Start the xChekPlus® software and log on.
2. Turn on the plate reader, and place the calibration plate in the holder.
3. From the File menu, select Template. Create a template for an assay you normally use, with a case name of your choosing and a count of 96. Save the template.
4. From the File menu, select Read.
5. Choose the assay and template you just created.
6. After the data is displayed, you may save it (using the save icon), and/or print it (using the print icon).
7. Using the printout, compare the optical density (OD) values for specific wells to the OD values on the calibration plate test card for the wavelength used.

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Best practices for using the IDEXX PRRS Oral Fluids Ab Test

- The IDEXX Porcine Reproductive and Respiratory Syndrome (PRRS) Oral Fluids Ab Test is easy to run; however, please keep the following in mind regarding sample collection and handling.
- Samples of oral fluids can be collected from individual pigs or from groups of pigs in pens using ropes that are hung at pig shoulder height.
- Ropes should remain in the pens for 20–30 minutes to ensure adequate exposure.
- Insert the wet end of the rope into a clean plastic boot or bag.
- Strip the rope so the fluid accumulates in the corner of the bag or flows into the tube, if the tube is connected to the bag.
- Cut a corner of the plastic bag and drain fluids into the collection tube. If using a bag with a connected tube, then disconnect the tube.
- A minimum sample of 2.5 mL is recommended. Make sure that collection tubes do not contain additives such as EDTA or heparin. If using glass blood collection tubes, use only red-top tubes (without additives).
- Discard all ropes after collection. Never reuse ropes, plastic bags or collection tubes. Never leave ropes in the pens after sampling is finished. Do not pool oral fluids samples from ropes in different pens.
- Be sure all tubes are tightly sealed and packaged with ice before shipping. Overnight delivery is preferred, as it ensures timely arrival of chilled samples for best results.
- If the oral fluid sample contains particulate from the mouths of the animals, the sample can be centrifuged at 2500g for 5–10 minutes before use.

Visit the idexx.com/prrs to view an informative video.

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Adjust your plate washer for best results

The plate washer plays a key role in achieving accurate, reproducible test results. Make sure your plate washer is adjusted properly. Change settings as needed so the washer meets these requirements:

- Buffer is dispensed gently, at approximately 200 µL/sec, with the tops of the aspiration pins level with the tops of the wells. Avoid bottom wash or similar modes. In bottom wash, the buffer is dispensed closer to the bottom of the wells, which increases the pressure on the well. There should be just enough pressure to clean the well.
- During aspiration, liquid is pulled from the surface of the wells, not from below or above. Synchronize the aspiration rate and the movement of the pins so that the pins always remain in contact with the liquid's surface as the level goes down. Use a high aspiration rate; the actual rate will vary depending on the washer. The aspiration pins should never be lower than 1 mm above the bottom of the wells.

Contact your local LPD Technical Services representative for any washer programming information available or any questions you may have.

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Understanding the ELISA certificate of analysis

IDEXX tests are manufactured in lots per strict quality standards. Lot approval by regulatory authorities, such as the USDA, is based upon the results of internal quality control testing for each. The certificate of analysis (C of A) for a test documents the performance of the test at the time of release testing. The performance data include the optical density (OD) values of the test controls and the calculated results and/or qualitative interpretation of a selected group of samples used during manufacturing. All these values must be within a specified range for the test lot to be approved for sale.

The OD values of the test controls do not need to remain within the C of A ranges during the lifetime of the test. Optical density values may change over time, without compromising test quality.

Diagnostic tests are composed of biological components that are not always stable when removed from their natural environment. Despite stabilizers used to control shelf life, most biological components lose some activity with time. When this occurs, OD values can decrease. The decrease in OD values of the test controls parallels the decrease of OD values in the tested samples, so the calculated results remain the same. Lower OD values than those on the C of A are acceptable so long as the validity specifications in the test insert are met for the assay.

The validity criteria in the insert differ from the values noted on the C of A. Validity criteria in the insert usually specify a limit on a control OD value or a ratio of control OD values. If a test plate performs within the validity specifications of the insert, then the test is working properly and the test results can be used.

The shelf life of an IDEXX diagnostic test can vary from 9 months to 2 years depending upon the product. The test is designed to give valid results throughout its shelf life.

With any ELISA, reproducibility and reliability depend on proper technique, equipment, environmental factors and attention to detail. The C of A serves as a guideline for anticipated test performance.

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Mixing samples for reliable test results

Mixing samples before plating is a very important step when running an ELISA test. Samples that are not mixed well will produce variable results.

Frozen samples can be thawed at room temperature or in a refrigerator. All thawed samples need to be thoroughly mixed prior to dilution to ensure that the proteins are dispersed throughout the sample. Mix by gentle vortexing or inverting at least five times. Frothing or overmixing of samples will cause denaturation of serum proteins.

Prepared dilutions, especially the 1:500 in poultry kits, also require mixing with gentle vortexing or a pipette set at a volume of 100 µl or greater prior to plating. Mixing the diluted sample with a micropipette, such as the one utilized for transferring the 1 µl of sample, is not appropriate as the volume exchanged is not enough to thoroughly mix the sample.

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How to ensure consistent performance with xChekPlus® software

When running ELISAs, a good measure of test performance is the reproducibility of the positive and negative control. xChekPlus software offers a report that displays not only data on the control replicates, but also control tracking over time.

It is important to review control data often to ensure that values are not trending or exhibiting excess variability. Control values for a specific lot should be similar on a run-to-run basis.

To run a control tracking report from xChekPlus:

1. Click on **Reports > Control Tracking Report.**
2. Highlight the test, specify a date, choose graphing options and check by well (% diff) > OK.
3. A control tracking report will be displayed that can be saved and/or printed and then evaluated.

Note: We recommend that the difference between control replicates be no greater than 20%.

A key component of the control tracking report is that it can display the lot number of the kit being used and represented in the control tracking report. This is useful information when evaluating quality control data. If you currently do not track the kit lot, it can be entered into the software in two ways: either through database > assays, or in plate view at the time that you are reading your plate.

If you are seeing poor control reproducibility, it may be an indication that your pipettes are not functioning properly, your wash system is malfunctioning, or you have poor distribution of antibodies in your samples.

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A review of ELISA formats

ELISA is divided into three main formats: indirect, blocking (competitive) and antigen-capture (direct).

Indirect Format

In the indirect format, the sample antibody is sandwiched between the antigen coated on the plate and an enzyme-labeled, antispecies globulin conjugate. The addition of an enzyme substrate-chromogen reagent causes color to develop. This color is directly proportional to the amount of bound sample antibody. The more antibody present in the sample, the stronger the color development in the test well. This format is suitable for determining total antibody level in samples (Johne's, etc.).

Blocking (Competitive) Format

In this format, the specific sample antibodies compete with, or block, the enzyme-labeled, specific antibody in the conjugate. The addition of an enzyme substrate-chromogen reagent causes color to develop. This color is inversely proportional to the amount of bound sample antibody. The more antibodies present in the sample, the less color development in the test well (CAV, etc.).

Antigen–Capture (Direct) Format

The antigen in the sample is sandwiched between antibodies coated on the plate and an enzyme-labeled conjugate. The antibody conjugate can be either monoclonal or polyclonal. The addition of an enzyme substrate-chromogen reagent causes color to develop. This color is directly proportional to the amount of the target antigen present in the samples (LLAg, etc.).

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Total Counts Report

The Total Counts Report provides the number of negative, positive, suspect, and total number of samples for each case selected. It also gives you a grand total for each category for all the cases selected. When creating the report, you can select the assay as well as the date range. The Total Counts Report allows a variety of uses. You may want to use it to determine a percentage of positive samples when testing presumed negative flocks or herds. You can also use the report to determine monthly or quarterly sample tallies for testing in your lab.

It is important to review control data often to ensure that values are not trending or exhibiting excess variability. Control values for a specific lot should be similar on a run-to-run basis.

To run a control tracking report from xChekPlus:

1. Choose Reports > Total Counts from the menu bar.
2. In the Total Counts dialog box, choose the assay and the data range (or leave the date range open to select all cases).

Note: You can also select the spreadsheet option if you want to save the data as a Microsoft® Excel spreadsheet.

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Pipetting to avoid bubbles

Careful pipetting is crucial in obtaining accurate test results when performing any ELISA test. Sometimes air, resulting in bubbles, can be drawn into the pipette or dispensed into the wells. If this happens, bubbles can influence optical density values and results. To minimize or eliminate this problem, reverse pipetting is recommended for the addition of reagents to the ELISA plate.

Reverse pipetting with a multichannel pipette:

1. Put new tips on the pipette, ensuring they are on tight and straight.
2. Press the plunger past the first stop and halfway to the second stop.
3. Draw the liquid in a slow motion, being careful that no air bubbles are drawn into the tip. Check for consistency of volume in the tips.
4. Touch the tips to the edge of the reagent reservoir to remove excess liquid on the outside of the tips.
5. If the wells on your plate are empty, position the tips into the lower corner of the wells.
6. If the wells on your plate contain liquid, position the tips above the liquid.
7. Slowly dispense the liquid into the wells by depressing the plunger to the first stop. Be careful not to splash liquid out of the wells, and make sure there are no drops left on the tips.
8. To repeat, hold the plunger at the first stop and continue with step 3.
9. Eject the tips into an appropriate waste container.

Note: Reverse pipetting uses more reagent/volume (=”dead volume”).

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Best practices for using the IDEXX Milk Pregnancy Test

Sample quality and handling

- The assay can be run on whole or skim milk samples and samples that have been exposed to heat treatment (during milk component analysis).
- Bronopol or a similar preservative may be used to maintain sample quality—this will not affect test results.
- Samples should be mixed gently prior to pipetting.
- Poor quality samples may compromise the accuracy of test results. Prior to testing, milk samples should be checked to ensure that they are not soured or separated and are free from contamination.
- Care should be taken to minimize the likelihood of milk carryover from cow to cow during sample collection, particularly when using samples collected for routine herd recording.

Incubation

- Use adhesive covers as specified during sample, detector and conjugate incubation steps. Adhesive plate covers validated for use with this test can be purchased from IDEXX. Ask your local sales or customer service representative for details.
- Use of a plate shaker incubator is needed. Please reference the test insert for rotation and temperature specifications or ask your local sales representative for more details.

Washing

- The assay requires 4–5 washes at each wash step during the assay protocol.
- Washing with good quality water and a clean and maintained washer is crucial.
- IDEXX can provide specific wash settings to be used for the IDEXX Milk Pregnancy Test. Please ask your LPD Technical Services representative for details.

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Unexpected positives

IDEXX ELISA screening assays are used to screen populations representing herds or flocks. Some negative populations may present with a higher number than expected rate of positives. Unexpected positives generated using any screening assay should be confirmed using an alternate confirmatory method appropriate for the test, such as Hemagglutination Inhibition (HI), quantitative polymerase chain reaction (qPCR), virus isolation (VI), etc.

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Intermixing reagents

IDEXX ELISA test kits are designed to work as a set of reagents. However, generic components, such as substrate, wash, and stop solutions can be mixed within kits having the same part number. Other reagents such as kit controls, conjugates and plates have been optimized to work together for that kit lot/batch. Should you have any questions on generic components or what can be used across kits, please contact your LPD Technical Services representative.

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