September 2025

Pathogen testing requirements for tomato and capsicum seeds

Information session – questions and answers

On 16 September 2025, we hosted two webinars to explain Australia's updated policies for Tomato brown rugose fruit virus (ToBRFV) and Tomato mottle mosaic virus (ToMMV) testing of tomato and capsicum seeds. These sessions also provided stakeholders with an opportunity to ask questions. A summary of the questions and answers from both sessions is provided below. Where required, additional content has been included to clarify the intent behind certain questions.

1. **Noting that laboratories must use 2 reverse-transcription qPCR protocols to verify the status of ToBRFV and ToMMV in each seed lot, can these 2 tests be done on the same sample?**

   Yes, both tests can be done using RNA extracted from the same subsample/sample and, in fact, each subsample/sample must be tested with the two tests.

2. **Does this test also cover other viroids?**

   At this stage, the only updated testing requirements apply to ToBRFV and Tomato mottle mosaic virus (ToMMV). These are the only tests that have changed recently.

   We do have existing testing requirements for several other tomato seed pathogens, including viroids, but there have been no updates to those protocols at this time. This may be reviewed in the future.

   Requirements for viroid testing remain unchanged and can be found in the department's BICON database.

3. **Do the tests need to be approved by the relevant exporting country's NPPO for the laboratory to be eligible to apply to Australia for approval?**

   This may be a requirement from the exporting country's National Plant Protection Organisation (NPPO), but it is not something the Australian government determines.

   When issuing phytosanitary certificates, the NPPO of the exporting country must acknowledge that the testing meets Australia's requirements. However, Australia does not require NPPOs to approve laboratories.

   The role of NPPOs in providing additional assurance may be considered in our future reviews. For now, NPPO approval of laboratories is not a requirement under Australia's current testing framework.

4. **Why was the introduction date set as November and not a more logical date of end of year when business close ?**
   We aimed to strike a balance between implementing the changes as soon as possible (so we could strengthen assurance in overseas laboratory testing) and allowing industry enough time to prepare.

   During the initial consultation, we received feedback that a shorter implementation period would be difficult for industry to meet. As a result, we chose a November start date to provide some flexibility without delaying the changes unnecessarily.

5. **Do we know if local labs capable of performing onshore testing will have increased capacity when this comes into effect?**

   There are currently two laboratories approved for seed testing in Australia, one at the Elizabeth Macarthur Agricultural Institute in NSW and one at the AgriBio facility in Victoria. The department has been engaging with both laboratories to ensure they are ready for implementation.

   To date, most seed testing conducted in Australia appears to be limited to small seed lots. If importers are planning to use Australian laboratories for testing, we encourage you to make early contact with the laboratories to provide advance notice.

6. **Will you still allow 20% tests?**

   Yes, there is no change to the current allowance for 20% seed sample testing for small seed lots. For standard seed lots, the requirement remains at 20,000 seeds.

   The only updates we've made relate to the PCR testing protocols for ToBRFV and ToMMV. All other virus and viroid testing requirements for tomato seed remain unchanged, including seed sample sizes, subsample sizes, and the 20% testing provision for small lots.

7. **Where can the Laboratory Registration form be found?**

   The Laboratory Registration form is on our Pathogen testing requirements for tomato and capsicum seeds webpage: agriculture.gov.au/biosecurity-trade/import/goods/plant-products/seeds-for-sowing/pathogen-testing-req-tomato-capsicum#labs

8. **It says the DAFF may conduct retesting of seed lots. What happens to this seed? Will DAFF pay for the testing and the cost of the seed?**

   Retesting by DAFF is not expected to be routine practice. If it does occur, it will be assessed on a case-by-case basis, depending on the specific circumstances and the justification for further investigation.

   If retesting is deemed necessary, DAFF will engage directly with the seed importer to determine the most appropriate approach, including discussions around costs and handling of the seed.

9. **How many offshore labs have approached the department for approval?**

   As of 29 September 2025, we have received 42 registration forms.

10. **Could a lab capable of conducting these new tests start tests now whilst preparing approval? Or would all tests needed to be started after the October approval date?**

    Yes, laboratories can begin testing now. In fact, we are aware that some laboratories may already be operating in line with the proposed conditions. In those cases, consignments tested by those laboratories may not require retesting and could continue to be traded.

We recommend discussing this directly with the laboratory you are working with to confirm whether their current practices align with the updated requirements and to ensure they are aware of what is expected.

11. **Will offshore countries recognise new protocols for testing and endorse phytosanitary certificates under new requirements?**

    We've received several queries directly from NPPOs, but so far, there's been no indication that any country intends to reject or refuse certification under the new protocols.

    To support international understanding and acceptance, we're also working through our Department of Foreign Affairs and Trade to communicate the changes, particularly to key trading partners.

    While we've received a few additional questions, there's currently no evidence that any country will be unable or unwilling to certify under the new requirements.

12. **With regard to onshore testing are you going to approve other labs?**

    Yes, we would certainly consider applications from additional onshore laboratories. Any lab, whether onshore or offshore, would need to meet the same standards and provide the same level of assurance under the authorisation process.

13. **DAFF recently acknowledged eradication of ToBRFV in Australia is not feasible. Can you explain some of the domestic movement requirements between Australian States? Where can this information be found? How was the ALOP determined for ToBRFV, there is a reference to a draft PRA, when will this be available for public review? Will the ALOP for domestic movement be the same as for international arrivals**

    The presenters today aren't the subject matter experts on this topic, but our colleagues in the department's Plant Health Policy area are actively working on it and coordinating discussions with state governments.

    Each state has its own biosecurity controls for domestic movement, which are outside the Commonwealth's authority. However, we support these efforts by applying international border requirements that help protect states where the virus is not present. For example, some states have confirmed detections of ToBRFV, while others are still free of the virus and have implemented movement restrictions to maintain that status.

    Our seed testing requirements reflect the current status of ToBRFV in Australia. ToBRFV is considered present with limited distribution and under official control, which means it remains a quarantine pest from a national perspective. This classification informs our international import requirements.

    These are complex issues involving multiple jurisdictions, and further updates will likely come from the Plant Health Policy area as the national management strategy evolves.

    For the most up-to-date information on domestic movement controls, you can visit:

    - Outbreak.gov.au
    - PIRSA,
    - Agriculture Victoria
    - NSW DPIRD.

14. How does that logic align for equivalency within the WTO and ISPM?

Within the WTO and ISPM frameworks, equivalence means that measures do not need to be identical, but they must provide the same level of protection. While individual states within Australia may implement slightly different approaches, these policies are assessed and endorsed nationally to ensure they collectively deliver the agreed level of protection. The department, as Australia's NPPO, is responsible for demonstrating this equivalence in our international arrangements.

15. Will the PRA's ever be made public around the ALOP determination?

At this stage, there are no plans to publish the Pest Risk Analyses (PRAs). However, this may be reconsidered in the future, particularly once there is a more consistent national approach to managing ToBRFV across states.

16. How many offshore labs were there recorded by the department previously?

We reviewed our records and identified approximately 20 offshore laboratories from which we had previously received testing documentation. As part of this recent review of testing conditions, we proactively reached out to these labs directly.

In addition to our public notifications, this direct engagement ensured that laboratories already involved in testing were aware of the updated requirements and had the opportunity to apply for authorisation if they wished to continue providing testing services.

17. Will a Turkish offshore lab be approved if they apply, given current issues?

During the investigation into the original outbreak, we identified seed imported from Türkiye that was positive for ToBRFV associated with one of the affected sites. Importantly, this seed has **not** been confirmed as the source of the outbreak.

This seed was certified by a Turkish laboratory as free from ToBRFV, but was later confirmed as positive through onshore testing in Australia. As a result, we made the decision to suspend acceptance of testing results from any laboratory in the Republic of Türkiye for seed imports into Australia. That suspension remains in place today.

We are actively working with Türkiye's NPPO to seek further assurance. This will help us assess whether Turkish laboratories may be authorised in the future, but for now, they are not eligible for approval under the current arrangements.

18. Comment from attendee: we've just done another import of tomato seed from turkey and preformed onshore testing and failed fyi

We know that we have a reasonable approach rate of seed that is infected with some of these viruses at the border, which underlines the importance of maintaining strong assurance around seed certification and testing.

To be clear, this issue is not limited to any one country, ToBRFV is known to be present across multiple regions globally. It's a widespread challenge for the industry, and our department remains committed to managing it responsibly.

Although ToBRFV has been detected in some Australian states, it is still considered to be under official control and limited to a small number of sites. Our goal is to minimise the impact of the virus within Australia by maintaining robust import conditions and supporting coordinated domestic management

19. **Are you going to be publishing a column with Protocol conditions like in the proposed testing requirements Table 1?**

    Authorised seed testing laboratories are responsible for determining the specific protocol conditions themselves, including the selection of optimal reagents, cycle times, and temperatures for each qPCR protocol. References to the relevant qPCR protocols are available at: agriculture.gov.au/biosecurity-trade/import/goods/plant-products/seeds-for-sowing/pathogen-testing-req-tomato-capsicum#reference-tables.

    This means we will not be prescribing detailed protocol conditions. We recognise that different laboratories may have specific reagents and conditions tailored to their own processes. Therefore, laboratories are free to optimise their own tests, including the choice of fluorescent labels for probes, under their own laboratory conditions. Once a list of approved laboratories is established, we may engage further with laboratories as needed, but initially, laboratories are not required to follow a single, prescribed protocol in detail.

20. **Are laboratories free to choose the fluorescent label for a probe?**

    Laboratories should use the fluorescent labels specified in our requirements, as these were used during protocol validation. If a different fluorescent label is preferred, please contact us to discuss approval.

21. **Can you provide examples of "anomalous" results?**

    There is no strict definition of an "anomalous" result, as this can vary between laboratories depending on their equipment, reagents, and standard practices. Generally, an anomalous result is one that does not meet the laboratory's usual expectations, such as a result that is below the cut-off but does not have a typical sigmoid curve. Laboratories are expected to use their professional judgement to identify and report such results. If a laboratory has questions about a specific test or result, they are encouraged to contact us for further guidance. Our guidelines have been updated to reflect this approach, and we aim to ensure consistency across laboratories.

    Note: If there are any concerns about technical details, such as primer sequences published on our website, please let us know so we can review and correct any errors as needed.

22. **If a seed lot tested under existing protocol is held up during shipping, and it goes beyond the implementation date, might it still be acceptable?**

    We recognise that there may be occasional consignments delayed in transit around the implementation date of 12 November 2025. These situations will be considered on a case-by-case basis. To help manage this transition, the list of approved laboratories will be published in advance, and new testing protocols will be accepted prior to 12 November 2025. If you anticipate that a shipment may arrive close to or after the transition, we recommend using the new testing protocols in advance to avoid delays.

23. **Subsample size is maximized to 400 seeds per subsample, is it possible for labs to increase subsample size to 1000 seeds per subsample?**

    A larger subsample size may increase the risk of false negative results, as it can reduce the sensitivity of the PCR test. The 400-seed limit is maintained as a general requirement. However, we are open to considering proposals for increased subsample sizes on a case-by-case basis following the current review. Laboratories seeking approval to use larger subsample sizes must submit a detailed proposal

to us. Please send requests via email to imports@aff.gov.au with the subject line 'Plant T2 - Seed for sowing'.

24. **In cases where we have already shown that our internal laboratory cut-off of 34 is as effective as the cut-off of 35, can we as well deliver the data to be able to use a cut-off 34 instead of 35?**

    At this stage, all laboratories are required to use the standard cut-off value of 35 to ensure consistency and comparability of results across all participating laboratories, regardless of any internal data supporting a different cut-off such as 34. This approach is necessary because we anticipate a significant number of laboratories may register for testing, and allowing individual variations would make it difficult to manage and compare results across the system.

    While we are not currently accepting proposals for alternative cut-offs, laboratories that have concerns about the suitability of the standard cut-off for their specific conditions are encouraged to contact us for further discussion. Our priority is to maintain a uniform approach during the initial implementation, but we may review this policy in the future as we gain more experience with the number and diversity of participating laboratories.

25. **Why are you not accepting testing the samples grey zone by the Bioassay method?**

    The bioassay method is not accepted for testing seed for freedom from disease and therefore cannot be used as evidence that seed is non-infectious. As a result, we do not use the bioassay method for any part of the testing process.

26. **Why have you removed the Levizky protocol for ToMMV?**

    The Levitsky protocol was removed because it is less sensitive than the approved qPCR protocols (such as Microlab). Additionally, several laboratories expressed a preference to move away from the Levitsky protocol and use qPCR methods for all tests, which supports a more consistent and sensitive approach. To ensure consistency and improve test sensitivity, the less sensitive endpoint PCR protocols, including Levitsky, were removed.

27. **Australia request labs a compromise for Cq of 35. Does this compromise apply for all tests realized in the labs, or only for those tests which are for export seeds to Australia?**

    The Cq (CT) cut-off of 35 is only required for the newly approved protocols for ToBRFV and ToMMV when testing seeds intended for export to Australia. This requirement applies specifically to Australia's import conditions for seeds entering the country from overseas. It does not apply to internal testing of seeds produced and moved within Australia, or to tests conducted for other purposes. Laboratories only need to apply the cut-off of 35 for tests performed to meet Australian import requirements.

28. **How does a laboratory engage with you to have a discussion?**

    Please submit requests via email to imports@aff.gov.au (please title the subject line of the email 'Plant T2 - Seed for sowing').

29. **In cases where a lab is already recognised by their NPPO, why do they need to register a laboratory again specifically for AU? Logically at the moment the NPPO from the export country is the one responsible to ensure that the seeds are tested according to the state of the art methods, and to a good quality level. What are the reasons to have the lab registering for the relevance of their tests again?**

We acknowledge that some laboratories are already approved and recognised by their National Plant Protection Organization (NPPO). However, this level of recognition and oversight is not consistent across all countries. Australia requires laboratories to register specifically for Australian import testing to ensure we have direct visibility of, and communication with, the laboratories conducting tests for our import conditions. This approach allows us to maintain consistent standards, improve communication, and address any issues directly with the laboratories involved. While we continue to review our processes and may consider additional assurances provided by NPPO recognition in the future, our current policy is to require direct registration with Australia to achieve the highest level of assurance and oversight for imported seed testing.

30. **If a seed lot was tested with Levitsky primers, negative, before the new rule (for example July 25), could we still organize the shipment of it after November?**

    Consignments that arrive in Australia on or after 12 November 2025 must meet the new testing requirements. If a seed lot was tested using the Levitsky protocol before this date and does not meet the new requirements, it will not be accepted upon arrival after 12 November 2025. Such consignments will be offered onshore testing at an approved laboratory in Australia (at the importer's expense) or will need to be exported or disposed of.

31. **Is the cut off the same for treated and untreated seeds? With no possibility to test if the virus is infective.**

    Yes, the same cut-off and testing protocols apply to both treated and untreated seeds. The department does not currently recognise any seed treatment to manage the risk of ToBRFV or ToMMV. Therefore, all seed lots must be tested according to the same protocols and cut-off values, regardless of any treatment applied to manage potential infection.

32. **Will you consider compiling Q&A based on these two information sessions?**

    Yes, we intend to compile a Q&A document based on the questions asked during these two information sessions. We will provide updated written responses and distribute them to attendees. Additionally, key questions and answers will be added to the department's website, where a list of frequently asked questions is already available under the emergency measures for Tomato Brown Rugose Fruit Virus. Please allow us some time to finalise and share these updated responses.

33. **Do we need to have both ToBRFV and ToMMV accredited by NPPOs to be able to register to become an authorised lab? For example, we are ToBRFV accredited but working on ToMMV currently while accreditation body is also working to publish a method for ToMMV.**

    Accreditation by your NPPO is a matter between your laboratory and the NPPO, and requirements may vary by country. For Australian import conditions, it is possible to register as an authorised laboratory for one test (e.g., ToBRFV) even if you are still working towards accreditation for the other (e.g., ToMMV). However, this means that seed lots requiring both tests may need to be tested at more than one laboratory. If you have concerns about your NPPO's recognition process or the timing of accreditation, please contact us directly. We are happy to discuss your situation and, if needed, liaise with your NPPO to help clarify requirements and avoid potential certification issues.

34. **Should there be additional control in case of treated seeds or disinfected seed? For example, inhibition control?**

    Laboratories are responsible for determining whether additional controls, such as inhibition controls, are needed when testing treated or disinfected seeds. If a seed treatment or coating could

potentially affect extraction efficiency or test performance, laboratories should consider implementing appropriate controls to ensure reliable results. While there is no specific requirement to add extra controls beyond the minimum standard, laboratories are free to include them as needed. If a laboratory has concerns that standard testing may not adequately address issues related to seed treatment, they are encouraged to contact us to discuss possible solutions.

35. **To be authorised lab, do we have only to fill the form? Or do we have to submit you validation report proving that we are able to do the testing and that we use your rules ?**

    At this stage, laboratories seeking authorisation are only required to complete and submit the application form. All necessary acknowledgements and details are captured within the form, and there is no current requirement to provide a separate validation report. However, we will continue to monitor the authorisation process and may request additional information or validation documentation from specific laboratories or all laboratories in the future if needed. For now, submitting the completed form is sufficient to be considered for authorisation.

    Further questions can be sent to imports@aff.gov.au Please title the email with 'Plant T2'.

36. **The Australian method requires CaTa28 and CSP as separate protocols. Imagine the Ct for CaTa28 is above 35 and CSP is 34.5. The second result is mandatory. Can we repeat this assay with CSP? Can we use the third marker, Menzel, to resolve the test? If we use the three markers and two are above 35 and one is between 34 and 35, what is the final result?**

    Yes. If a result appears anomalous, the laboratory may either repeat CSP on the same subsample or use Menzel as the second approved protocol. Only the approved assays (CaTa28, CSP1325, and Menzel) may be used. A Ct ≤ 35 is positive, >35 is negative, and the lowest Ct across replicates is used for interpretation. Therefore, if two replicates are >35 and one is 34.5, the subsample is considered positive, unless retesting with the same approved protocol demonstrates the result was anomalous. Full details are available in the department's guidance on Ct cut-offs and interpretation requirements