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| RESEARCH ARTICLE

Advances in Molecular Diagnostics and Biosensor Technologies for Potato Virus Y (PVY): Strategic Integration into U.S. Seed Certification and Precision Agriculture Systems

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| ABSTRACT

Potato virus Y (PVY) is a positive-sense single-stranded RNA virus in the Potyvirus genus (family Potyviridae). It is still one of the most important viral factors that limit potato production and is one of the main reasons why seed lots are downgraded or rejected in modern certification systems [1,2,3]. Over the last thirty years, the study of PVY epidemiology has changed from the traditional "ordinary" PVYo populations toward genetically complex recombinant lineages (e.g., PVYN-Wi, PVYNTNa, PVYN:O) that are often linked to tuber necrosis phenotypes and are harder to figure out using only old serological typing [1,2,3,4]. This change is happening because PVY has a strong ability to evolve. This is especially true in vegetatively propagated seed systems, where infection can build up over generations and recombinants can spread quickly once they are established [1,2,4]. Even though ELISA and RT-qPCR are still the most important tests for regulatory purposes, they are becoming harder to use because of the need for (i) higher analytical sensitivity in dormant tuber matrices, (ii) better differentiation of recombinant strain groups, and (iii) decentralized testing to speed up the time it takes to make a decision about seed lot certification. New nucleic acid platforms like RT-LAMP, RT-RPA, CRISPR-Cas assays, digital PCR, and high-throughput sequencing (HTS) are making it possible to achieve higher levels of sensitivity, portability, and sequence resolution [6,7,8,9,10,11,12]. At the same time, biosensor technologies like surface plasmon resonance, quartz crystal microbalance, electrochemical nano/graphene sensors, and aptamer-enabled devices are coming together with molecular recognition chemistry to make "sample-to-answer" formats that can be used in the field [13,14,15,16,17]. Adding these diagnostic outputs to precision agriculture tools like IoT dashboards, remote sensing, vector forecasting, and disease risk modeling can help contain problems sooner and make certification actions more defensible [18,19]. This review brings together information about PVY molecular evolution and recombination biology, focusing on what it means for seed certification in the U.S. It then looks at next-generation diagnostic methods through a translational lens, focusing on analytical performance, deployment feasibility, strain resolution, and standardization requirements. It also suggests an integrated surveillance framework that connects advanced detection to certification policy, agricultural biosecurity, and national food system resilience.

| KEYWORDS

Potato virus Y; recombinant strains; seed potato certification; RT-qPCR; CRISPR-Cas diagnostics; isothermal amplification; biosensor platforms; high-throughput sequencing; precision agriculture.

| ARTICLE INFORMATION

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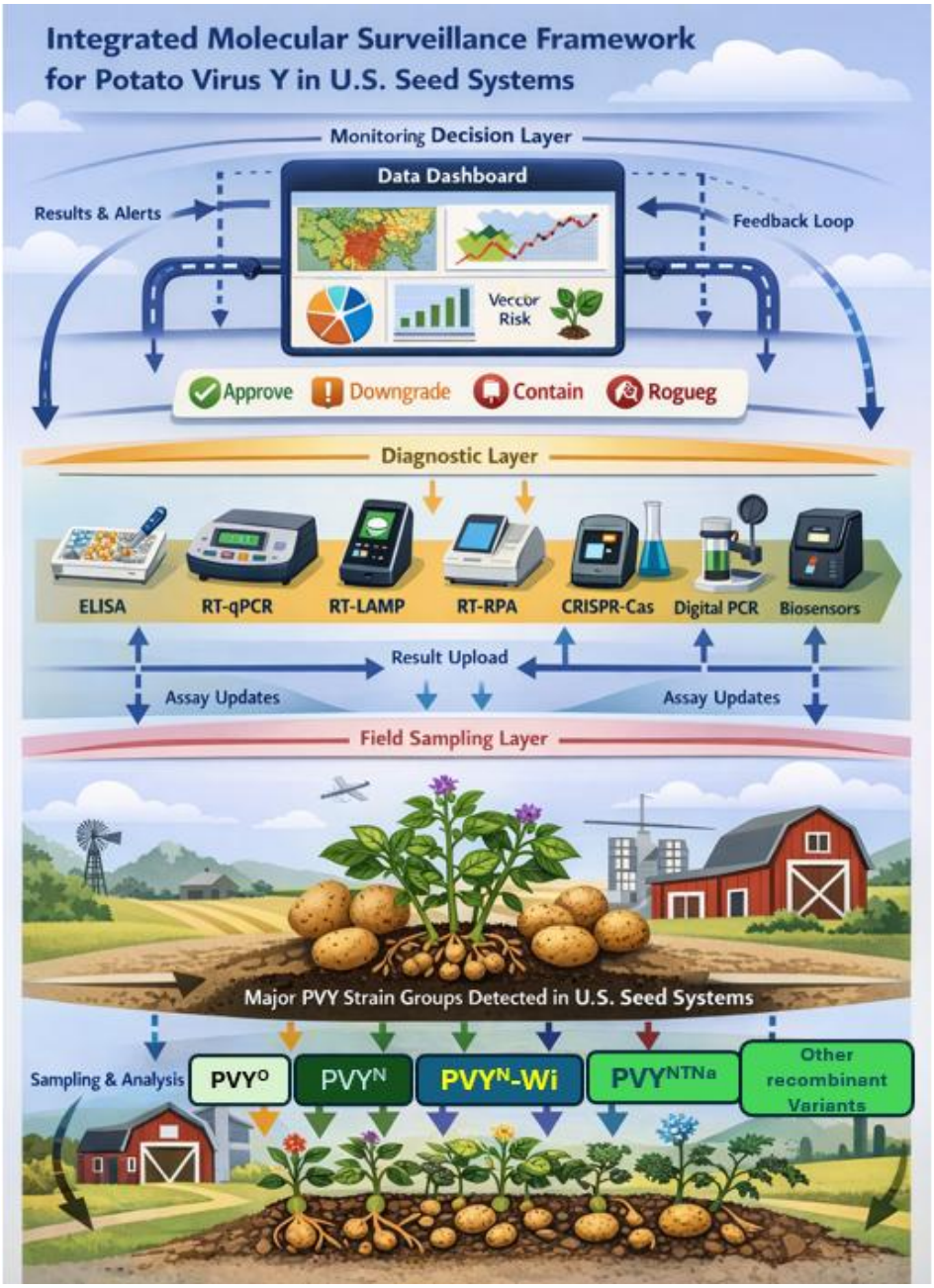


Graphical Abstract: Integrated Molecular Surveillance Framework for Potato Virus Y in U.S. Seed Systems. The graphical abstract shows a tiered PVY surveillance ecosystem that links biological diversity to certification decisions that can be acted on. **Bottom layer:** shows that infected plants and seed tubers have a range of PVY strains (PVY^{O}, PVY^{N}, PVY^{N}-Wi, PVY^{NTNa}, and more recombinants). This is because of the population structure and strain replacement that have been seen in North American seed systems [1,4]. **Middle layer:** Diagnostic workflows are arranged from centralized to field-deployable conventional ELISA and RT-qPCR (regulatory baselines) alongside RT-LAMP, RT-RPA, CRISPR-Cas detection, digital PCR confirmation, and biosensor formats (electrochemical/graphene, QCM/SPR) [5,6,7,9,10,11,12,13,14,15,16,17]. **Top layer:** Results are sent to decision dashboards through IoT-enabled reporting. These dashboards combine epidemiological modeling and vector risk to help decide whether to accept, downgrade, contain, or target rogueing a seed lot. They also help prioritize confirmatory sequencing when recombinant signatures change [6,10,18,19]. The diagram shows that feedback goes both ways: surveillance data updates primer/probe sets and guide RNAs, and certification outcomes affect sampling design and risk thresholds.

1. Molecular Virology and Evolutionary Dynamics of PVY

1.1 Genome Architecture and Protein Function

PVY has a positive-sense RNA genome that is about 9.7 kb long and codes for one polyprotein that is cut into functional products, which is a common trait of potyviruses. The genome has a 3' poly(A) tail and is connected to VPg at the 5' end [2]. The processing of polyproteins produces proteins that control replication, movement, and transmission. These proteins include HC-Pro (a multifunctional helper component proteinase), CI helicase, NIb RNA-dependent RNA polymerase (RdRp), and the coat protein (CP) [2,20]. HC-Pro is especially important because it helps aphids spread disease and is a major RNA silencing suppressor. It connects how well an infection spreads within a host to how well it spreads across a population [20,21,22]. Like other RNA viruses, error-prone RdRp replication helps subspecies diversity, which lets them adapt quickly to selection caused by host genotype, vector ecology, and certification-driven bottlenecks [23].

1.2 Recombination and Strain Diversification

Recombination is the main way that evolution happens in the PVY complex, and it has shaped the structure of modern strains and the complexity of diagnostics. Recombination breakpoints tend to happen in certain areas (like the P1/HC-Pro and NIb/CP junctions), which makes mosaic genomes that can change the way symptoms show up, the way antigens work, and the way assays bind and primers match [1,2,4]. Strain groups within the PVY^{N}-derived recombinant spectrum have been linked to recombinant-associated tuber necrosis phenotypes, such as PTNRD [3,4]. Longitudinal data from U.S. seed production show a major strain replacement: the prevalence of PVY^{O} has dropped sharply, and by the early 2020s, recombinants (especially PVY^{N}-Wi and PVY^{NTNa}) will make up the vast majority of PVY positives in key seed-growing areas [4]. This makes (i) designing assays that are aware of recombination and (ii) doing whole-genome surveillance on a regular basis more important to stop diagnostic drift.

2. Epidemiology and Transmission Biology

Many aphid species spread PVY in a way that doesn't last long and doesn't go around in circles. The virus is taken in within seconds to minutes during probing, is kept for a short time, and is then passed on quickly before insecticides have a significant effect on the chances of passing it on [21,22]. As a result, managing PVY relies more on clean seed, keeping an eye on vectors, field hygiene/rogueing, and timing than on chemicals alone. Temperature-driven aphid dynamics and mixed infections that may change how the virus replicates and how symptoms show up affect epidemiological outcomes [1,3].

Remote sensing has become an additional layer for detection. When combined with deep learning, hyperspectral imaging can tell the difference between PVY-infected plants and healthy controls. When used with molecular confirmation, it can also help with earlier or more objective field scouting [18].

3. Economic Modeling and Agricultural Impact

PVY lowers yield and market value by making tubers smaller, lowering their quality, and killing them in susceptible cultivars. This has a bigger economic impact on seed systems because the infection spreads from generation to generation and can cause certification rejection or downgrade [1,3,4]. PVY is often cited as a major reason for seed lot rejection or downgrading in the United States. This is because it causes biological damage and enforces regulatory thresholds [4]. The practical implication is that diagnostics are an economic control lever: earlier detection and better strain discrimination lower the chances that infected seed will move through distribution, which stops the loss from getting worse.

4. Quantitative Molecular Diagnostics: RT-qPCR and Digital PCR

4.1 RT-qPCR as the Current Regulatory Baseline

RT-qPCR is very sensitive and is often used to find PVY in a wide range of samples, such as tubers, leaves, and dormant tuber peel extracts [5,6]. RT-qPCR is better than ELISA at finding low-titer samples and supports strain-aware targeting when tests are made to cover conserved regions or strain-discriminatory motifs [5,6]. The implementation of large-scale certification shows that it is possible to operate: high-throughput RT-qPCR pipelines can cut down on cycle time by allowing direct testing of dormant tubers and reducing the need for workflows that break dormancy [6].

4.2 Multiplexing and Strain Typing

Since recombinants make up most of today's PVY populations, multiplex molecular assays that can find and tell the difference between strain groups (including recombinant signatures) are very useful. A fundamental multiplex RT-PCR framework for PVY strain characterization demonstrated the viability of single-assay classification of predominant strain types and mixed infections, an approach that continues to be conceptually significant for contemporary multiplex RT-qPCR and CRISPR guide design [24].

However, ongoing recombination and population shifts necessitate continuous primer/probe auditing against current genomes, ideally augmented by periodic HTS surveillance [1,4,10].

4.3 Digital PCR (ddPCR) for Confirmatory Testing

Digital PCR allows for absolute quantification by breaking reactions up into many microreactions. This makes it more accurate when there are few target copies and less sensitive to inhibitors [25]. Even though ddPCR research is more general for plant pathogens, it is directly related to PVY certification as a way to confirm high-value lots or results that don't match, especially when tuber matrices have inhibitors that can make regular qPCR more difficult [25].

5. Isothermal Amplification Platforms

5.1 RT-LAMP

LAMP uses 4–6 primers that recognize different target regions and work at a constant temperature. This makes the hardware simpler and speeds up the time it takes to get results [7]. PVY-specific RT-LAMP has been shown to work on plant samples, which supports the idea of field-forward testing (colorimetric readouts, closed-tube formats) [8]. Important translational limitations are the difficulty of designing primers, the need for a false positive control, and the need for standardization for validation at multiple sites.

5.2 RT-RPA

RPA is a type of isothermal amplification chemistry that works at low temperatures and is powered by recombinase-primer complexes and strand displacement polymerase. It usually works near body temperature and gives results quickly [9]. Multiplex RT-RPA with lateral-flow detection has been shown to work for potato viruses like PVY. This shows that multi-target panels (PVY + PVS + PLRV) can be made in about 30 minutes with very little equipment [11]. These traits work well with on-farm triage and decentralized seed lot screening, especially when they are used with confirmatory lab testing.

6. CRISPR-Cas–Mediated Detection

CRISPR diagnostics use guide RNAs to find target sequences and turn on collateral nuclease activity (Cas12 for DNA-linked workflows and Cas13 for RNA targets). This makes it possible to generate signals using fluorescent or lateral-flow reporters [26,27]. The SHERLOCK paradigm (Cas13-based detection) is especially appealing for RNA viruses such as PVY when combined with pre-amplification techniques (e.g., RPA) to enhance sensitivity [26]. Plant-virus-focused CRISPR diagnostic reviews stress making things more possible, such as field-forward formats and strain-aware design strategies [12].

For PVY, the most valuable application is recombinant/strain discrimination. Guide RNAs can be made to target strain-specific motifs or recombination junctions. This gives results that are hard to get reliably with serology and is becoming more important in U.S. seed systems that are mostly recombinant [1,4,12].

7. High-Throughput Sequencing for Surveillance

HTS offers genome-wide resolution, including full-genome reconstruction, recombination mapping, minority variant detection, and mixed infection characterization, which helps with both outbreak investigation and proactive assay updating. HTS is becoming more and more seen as a strategic surveillance layer that works with targeted assays. It helps redesign primers and probes, confirms new recombinants that might not be picked up by older assays, and gives solid proof for policy changes in certification programs [10,28,29].

8. Biosensor Platforms for PVY: Engineering Principles and Translational Potential

8.1 Surface Plasmon Resonance

SPR lets you see biomolecular interactions in real time without using labels. It is often used to characterize antibodies and profile their kinetics. SPR monitoring of the interaction between PVY antibodies and viruses helps with assay development and biosensor proof-of-concept, but it can't be used in the field because the instruments are too complicated and the matrix interferes with crude plant extracts [13].

8.2 Quartz Crystal Microbalance (QCM)

When binding happens at the sensor surface, QCM picks up changes in mass on a vibrating quartz crystal. QCM immunosensors have been used to find viruses in plants, which shows that label-free capture-based biosensing is possible [14,15]. For PVY, QCM

is best seen as a laboratory development platform (bioreceptor optimization, matrix evaluation), and making it portable will take more engineering work.

8.3 Electrochemical and Graphene-Enabled Biosensors

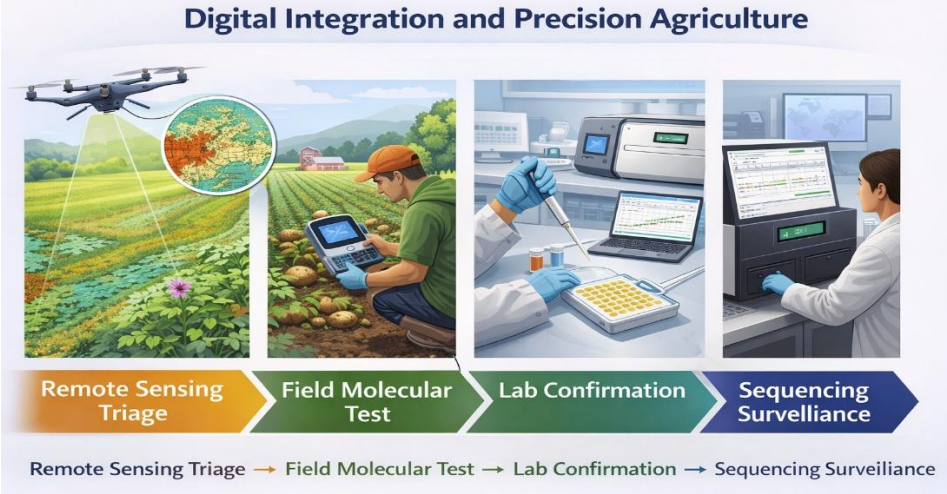
Electrochemical biosensors convert binding or nucleic acid hybridization events into electrical signals (impedance/current changes) and can be used in small, low-power devices. Graphene and similar nanomaterials are good transducer substrates because they are conductive and can be modified on their surfaces. This makes them useful for portable pathogen detection [17]. When used with strong sample prep and standard thresholds, these formats work very well with smartphone readers and IoT dashboards.

8.4 Aptamer-Enabled Biosensing

Aptamers are synthetic recognition elements that can be used instead of or in addition to antibodies. They often make things more stable and make it easier to make things in a standard way. A lot of reviews of broad aptamer biosensors show that they are useful for finding pathogens and that they work well with electrochemical transduction [16].

9. Digital Integration and Precision Agriculture

When PVY diagnostics are built into decision systems, they are most useful. For example, geo-tagged results, time-stamped outputs, and standardized reporting can help with risk mapping, targeted rogueing, and certification decisions. Hyperspectral imaging combined with deep learning can pinpoint potential infection zones for confirmatory testing, enhancing sampling efficiency, minimizing labor, and elevating the likelihood of early detection [18]. This multi-step process (remote sensing triage → field molecular test → lab confirmation → sequencing surveillance) is a realistic way to move forward.



10. U.S. Seed Certification: Regulatory Integration Requirements

Emerging platforms must show the following to be used in certification workflows:

1. Sensitivity and specificity that are like those of validated RT-qPCR baselines
2. Reproducibility across laboratories and field environments
3. Robust performance in relevant matrices (dormant tuber peels, sprouts, leaves)
4. Validated coverage against current recombinants [4,6,10].

A practical adoption pathway is "tiered validation": It means testing it against current standards in parallel, running ring trials in different states and labs, and adding it to guidance documents once its performance and operational feasibility have been shown.

11. Discussion: What Evidence Means for PVY Surveillance and Certification

The PVY diagnostic landscape is experiencing a transformation similar to that observed in human infectious disease testing: a shift from centralized, laboratory-dependent workflows to tiered, risk-based testing frameworks that integrate rapid screening with high-confidence confirmatory techniques. In PVY, this change is not up for debate. It is necessary because many North

American seed systems are dominated by recombinant lineages, and diagnostic performance must keep up with strain turnover and recombination-driven sequence drift [1,4,30].

11.1 Why recombinant PVY changes the "diagnostic definition" of success

In traditional seed programs, "PVY positive vs negative" might have been enough to make decisions about how to run things. But in modern systems, "PVY positive" can mean strain groups that have different effects on tuber quality, symptom expression, and economic consequences (for example, necrotic phenotypes). Recombinant-associated variants make it harder to tell different serological tests apart and can cause primer/probe mismatches as populations change. This raises the chance of false negatives if tests aren't updated regularly [1,2,4,31]. Because of this, surveillance needs to be aware of strains and able to change.

11.2 RT-qPCR remains essential, but not sufficient alone

RT-qPCR is still the current regulatory baseline because it can find things very accurately, handle a lot of samples at once, and work with laboratory quality assurance and quality control systems [5][6]. RT-qPCR is not inherently "future-proof" against PVY recombination: primers and probes must be audited against contemporary genomes, and certification programs must incorporate periodic sequence-informed redesign [1,4,10,32]. Centralized qPCR pipelines are also prone to seasonal bottlenecks, such as sample surges and reagent logistics. This is why field-forward screening tools are needed to ease the load on reference labs without lowering confidence.

11.3 Field-forward methods are best positioned as screening layers

Isothermal amplification (LAMP and RPA) works well for decentralized sampling situations like field inspections, grower triage, and pre-lot screening because it doesn't need as much equipment and can be done quickly [7,8,9,11]. But we should be realistic about the operational role of these tests: they are most useful as screening tools that help us figure out which lots need confirmatory RT-qPCR or sequencing, not as replacements for regulatory baselines right away. This is how other regulated testing systems use quick tests.

11.4 CRISPR diagnostics provide the clearest path to "strain-aware field testing."

CRISPR-Cas detection has a special feature: programmable recognition that can be set up to find strain-specific motifs or recombinant junction regions [12,26,27]. CRISPR can, in theory, connect the speed of field-deployable tests with the accuracy of lab-grade tests, especially when used with RPA [26,27]. For PVY, where recombination is a big part of the epidemiological risk, CRISPR is very useful because it can directly encode strain resolution into assay design.

11.5 HTS is not routine testing, but it is the surveillance backbone

HTS is not likely to become a standard certification test anytime soon because it costs too much, is too complicated to use, and requires bioinformatics [10,28]. However, HTS is an essential part of surveillance because it:

1. keeps an eye on PVY strain turnover,
2. finds new recombinant variants early, and
3. keeps all molecular platforms up to date on primer/probe/guide changes [1,4,10,28].

Instead of trying to sequence everything, a better way to do things is to periodically sample representative seed lots and regional hotspots.

11.6 Biosensors are promising, but sample prep and standardization remain the bottleneck

Biosensor technologies (SPR/QCM/electrochemical/graphene/aptamer-based systems) are fast and easy to move around, but they have two big problems when it comes to translation:

- the complexity of the sample matrix (plant sap inhibitors, debris, and changing viral titers), and
- a lack of standardized validation frameworks across labs and certification bodies [13,14,15,16,17].

Electrochemical sensors are the easiest to use on farms because they use little power and work with portable readers. However, they will need strong front-end sample preparation and field validation against RT-qPCR baselines [16,17].

12. Practical Implementation Roadmap for U.S. Certification Programs

A tiring, risk-based surveillance framework is a possible way to modernize:

Tier 1 — Field Screening (fast, low-cost):

- RT-RPA-LFD panels for PVY and other potato viruses that are also present [11].
- RT-LAMP for quick screening of PVY presence [7][8].
- Early-stage CRISPR-RPA for strain-aware detection as validation matures [12, 26].

Tier 2 — Certification Laboratory Confirmation:

- Using RT-qPCR as the basis for certification decisions [5,6].
- Digital PCR for settling disagreements, confirming low titers, or working with matrices that have a lot of inhibitors [25].

Tier 3 — Genomic Surveillance and Assay Updating:

- Regular HTS sampling to find recombinants and change the targets for primers, probes, and guides [10,28].

This method keeps regulatory defensibility while making things faster, more scalable, and more resilient during busy times.

13. Limitations and Standardization Gaps

For widespread use, a number of problems need to be fixed:

1. **Inter-laboratory comparability:** standardized controls, reporting thresholds, and proficiency testing [6,10].
2. **Matrix performance:** confirmed protocols for dormant tuber peels, sprouts, and leaves [6].
3. **Strain coverage:** regular updates of molecular targets using databases that are based on high-throughput screening [1,4,10].
4. **Contamination control for isothermal methods:** closed-tube detection, workflow compartmentalization, and audit trails [7,8,9].
5. **Regulatory acceptance:** evidence packages that show that RT-qPCR is equal to (or better than) other methods in the same field conditions [6].

14. Future Research Directions

14.1 Fully Multiplexed, Strain-Aware Field Diagnostics

Because PVY is so common in U.S. seed systems [1.4], future diagnostic development needs to go beyond single-target detection and create fully multiplexed panels that can:

- The ability to find PVY and other potato viruses that are circulating at the same time (PLRV, PVS, PVA)
- Distinction among PVY recombinant lineages
- Finding signs of recombinant junctions

This evolution is best suited for CRISPR-Cas systems. When combined with RT-RPA amplification, guide RNA multiplexing makes it possible to programmatically tell the difference between strain-defining motifs [12.26].

Future research ought to concentrate on:

- Libraries of recombinant junction-specific guides
- Reducing cross-reactivity in multiplex settings
- Validation in the field in the main potato-growing areas of the U.S.

This method would greatly cut down on diagnostic blind spots that are caused by primer mismatches or antigenic drift.

14.2 Integrated RT-qPCR HTS Adaptive Surveillance Pipelines

RT-qPCR tests need to be checked against the genomes that are currently circulating on a regular basis. A surveillance model that looks ahead includes:

1. Regular RT-qPCR certification testing [5,6].
2. Targeted HTS surveillance sampling during each production season [10, 28].
3. Automated bioinformatic screening for primer/probe mismatch
4. Updating diagnostic designs in real time

Using artificial intelligence to help find recombinant breakpoints could speed up the process of finding new lineages before they become widely established.

These adaptive pipelines change surveillance from being reactive to being proactive.

14.3 CRISPR–Electrochemical Hybrid Platforms

New CRISPR-electrochemical integrations use impedance-based or redox signal outputs instead of fluorescence. This makes it possible to detect things with a battery-powered smartphone [16,26].

Future engineering priorities are:

- Stability of lyophilized reagents (4–40°C tolerance)
- Cartridges for preparing microfluidic samples
- Control of contamination in closed cartridges
- Cost targets for farm adoption are less than $8 per test.

This type of platform has the best long-term potential for decentralized seed screening.

14.4 Remote Sensing Coupled with Molecular Confirmation

Deep learning and hyperspectral imaging can find possible infection zones before symptoms show up [18,19]. However, molecular testing must confirm spectral signatures to prevent false positives.

A model that can grow is:

Remote sensing triage → targeted RT-RPA/LAMP → laboratory RT-qPCR confirmation → periodic HTS surveillance.

This layered model makes it easier to sample while also increasing the chances of finding something early.

14.5 National-Scale Data Integration and Decision Dashboards

IoT-enabled diagnostic nodes might send:

- Infection data with GPS tags
- Estimates of viral load
- Classifying strains

Combining aphid migration models with climate data can make PVY risk maps that can be used to make predictions.

These kinds of digital ecosystems make things better:

- Targeted rogueing
- Keeping seed lots separate
- Defensibility of export certification

15. Substantial National Importance

Potato is one of the most important specialty crops in the US economy. It is the basis for both domestic and international markets. Maintaining varietal purity, yield stability, and phytosanitary credibility in global trade depends on the integrity of seed certification.

Potato virus Y is the most dangerous virus for certified seed systems because:

- Its prevalence throughout U.S. production areas [4].
- Recombinant-driven strain diversification [1,4].
- Recorded effects on tuber quality and seed lot demotion [3].
- Fast spread through aphids [21].

Not updating PVY surveillance makes it more likely that:

- Rejection of seed lot
- Spread of recombinant strains between states
- Problems in the export market
- Less stable yields

Advanced strain-aware diagnostics that work with precision agriculture directly improve:

- Food security in the U.S.
- The ability of the agricultural supply chain to bounce back
- The ability to compete in exports
- Getting ready for biosecurity

The suggested combination of CRISPR-based detection, adaptive RT-qPCR surveillance, and digital certification pipelines addresses a major weakness in the agricultural sector in the United States.

16. Proposed Endeavor Statement

Proposed Endeavor:

Creating and combining strain-aware, field-deployable molecular tests for Potato virus Y to make U.S. seed certification, agricultural biosecurity, and the supply chain stronger.

This project will:

1. Make multiplex CRISPR-based PVY detection systems that focus on junction regions that have been recombined.
2. Make portable diagnostic tools that use both RT-RPA and electrochemical biosensor technologies for decentralized seed screening.
3. Create adaptive surveillance pipelines that link RT-qPCR certification testing with regular high-throughput sequencing for monitoring strains.
4. Make digital reporting systems that can combine geo-tagged infection mapping and risk modeling.

The goal is to make a multi-tiered diagnostic ecosystem that can grow, and that helps find problems early, speeds up certification, and keeps the spread of recombinant PVY from hurting U.S. potato production.

17. Conclusion

PVY has become a viral complex dominated by recombinants, which has a big effect on potato yield, tuber quality, and the integrity of seed certification. [1,4]. This change makes it more likely that old workflows, especially those that depend on static serological discrimination or primer designs that haven't been updated, will not work as well as populations change. [1,2,10]. RT-qPCR is still the most important part of certification because it is sensitive and can be used on a large scale. However, the next generation of PVY control will need a tiered system that includes field-forward screening (LAMP/RPA), strain-aware specificity (CRISPR), and genome-scale surveillance (HTS) [5,6,7,8,9,10,11,12,26,27,28].

From an implementation standpoint, modernization is most feasible through incremental validation and incorporation into existing certification frameworks: initially as a screening mechanism, subsequently as an endorsed confirmatory instrument once multi-site validation evidence consistent performance in pertinent matrices and against current recombinants [4,6,10]. Over time, integrating with precision agriculture data streams like remote sensing, vector forecasting, and geo-tagged diagnostics can change how PVY is managed from reactive containment to predictive prevention. This will make U.S. agriculture more resilient and the supply chain more reliable [18,19].

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