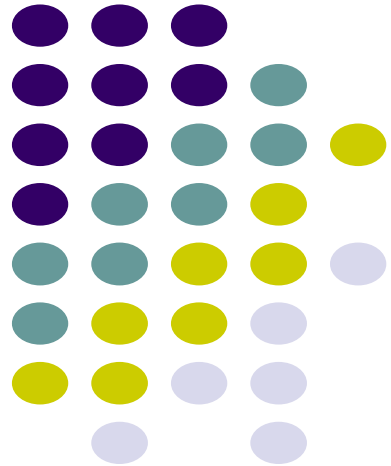
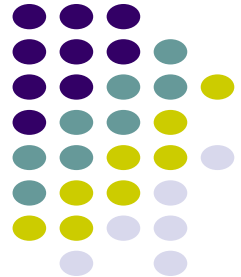
On-Site Testing: Moving Decision Making from the Lab to the Field



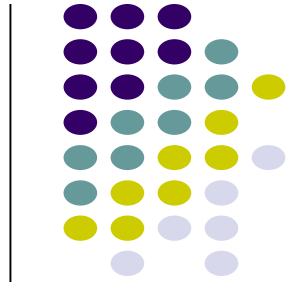
- ‘On-site testing’ is a term that is often used to describe two distinct activities,
  - Firstly detection is the initial locating of the pest or pathogen infected sample which in most instances is performed visually.
  - The second activity is identification, usually this is achieved by sending suspected samples to a laboratory.
- Providing technological solutions to enable more rapid decision making is a must nowadays. It is not necessarily just inspection services who benefit from these techniques, they can be deployed throughout the farm to fork to limit losses caused by pathogens.
- How best to deploy detection methods is however a matter for policy makers and other stakeholders. Deploying simplified detection and identification methods remotely helps to speed up inspection and facilitates trade.



- Performing diagnostics is part of a decision making process to prevent or limit spread of pathogens. The faster the decisions are made more effective the action may be.
- Traditionally once a potential disease has been located, samples are sent to a laboratory for testing that causes delay in the decision making process. Furthermore, If pathogens go unnoticed at pre-symptomatic infection stages, this failure of visual observation can lead to its spreading unchecked until it has built up to such a level that it can be seen.
- These issues have led the drive to develop technological solutions that would fulfil two complementary roles.
  - Firstly, putting tools into the hands of those on the front line to enable rapid identification of pathogens would prevent delays.
  - Secondly, developing detection tools that guide those on the front line to the site of the problem, at the pre-symptomatic infection.
- These tools when linked together enable a more efficient detection and diagnosis process enabling faster deployment of control measures.

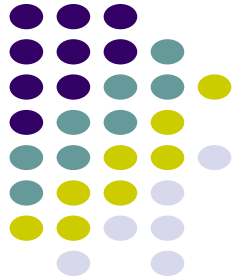
- Methods based on latex agglutination have been performed for plant diseases since the early 1980s (e.g. Potato virus test kits by Ani Biotech)
- Since then more refined methods have been developed to enable rapid identification. Early test kits based on latex agglutination on glass or plastic slides, required:
  - a large number of temperature labile reagents,
  - had multiple steps in which reagents were added sequentially and
  - the interpretation of the result was often subjective, requiring a fair amount of training and experience to reproduce.
- Some of these second generation kits (e.g. Alert kits by Neogen) also incorporated chemical substrates, effectively recreating laboratory ELISA methodology, yet performed rapidly on a solid support. This provided advantages in terms of both usability and interpretation of results which were no longer subjective and easily interpreted by non-specialists in a field.

- The most significant innovation came in the late 1990s with the application of homogeneous test kit formats developed and exploited.
- The Lateral Flow Device (LFD) format was exploited initially in the phytodiagnostics arena for the detection of potato viruses for use in seed certification systems and proved to be a considerable improvement over previous formats. The underlying chemistry in an LFD is effectively the same as a latex agglutination kit, the accumulation of antibody coated latex (or colloidal gold) particles caused by the presence of the antibody target.
- The key difference however is that the binding occurs during the capillary flow of sample and reagents along a membrane, rather than in solution.

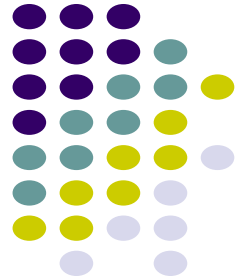


- Testing based on LFD technology remains the simplest and most rapid option for field use where specific binding reagents for the targets of interest are available.
- The only significant drawbacks to LFD approaches to field detection are the availability of reagents with a specificity appropriate for the application and the inherent lack of amplification that limits sensitivity.
- For simple pathogens such as viruses and to some degree bacteria and fungi, antisera or monoclonal antibodies with a useful level of specificity are often available, but for more complex targets this is often not the case.

In-Field Identification



- For greater sensitivity and specificity, molecular biology methods such as PCR is used to amplify target nucleic acids. However, implementation of these methods on-site has been a challenge.
- A number of companies have produced fieldable real-time PCR equipment.
- Whilst portable real-time PCR has been evaluated extensively there are a couple of significant drawbacks to it implementation.
  - Firstly, in PCR methods, extraction of nucleic acid generally requires reasonably elaborate extraction methods to avoid co-purification of compounds which inhibit the enzymes.
  - Secondly, whilst rugged, portable and in some cases battery powered equipment is available it remains expensive, largely due to the need for careful temperature regulation and sensitive detectors are needed
- To solve both of these problems subsequent research has been focused on evaluation of isothermal amplification chemistries.



- Isothermal amplifications are methods in which the amplification reaction is incubated at a single temperature. This gives advantages in terms of simplicity over PCR, since the reactions do not need to be cycled accurately between temperatures, thus water-baths, dry-blocks or incubators can be used to incubate reactions.
- Plant pathogen assays by the Loop mediated AMPlification (LAMP) method is the most widely adopted method to date.

Finding Pathogens

- Sniffing Pathogen Infection
- Seeing Infection from a Distance
- Use of Surveillance Tools