Research on Diagnostic Tests: Key Topics and New applications

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Atomic energy for peace, health and prosperity

Sustainable agricultural development, improved nutrition and food security

(1962)

to contribute to sustainable food security and safety by use of nuclear techniques and biotechnology

Joint FAO/IAEA Programme in Food and Agriculture
Corporate Mission
Historical Serological tests for disease confirmation are:

- Seroneutralisation test
- Complement fixation test
- Agar Gel Immunodiffusion test

For these tests often it was recommended to have pair test sera collected at least 15 days apart. Such a recommendation and the difficulties to isolate some pathogens are not compatible with effective management of diseases able to spread quickly such as transboundary animal diseases.
Traditional Technique of Disease Diagnosis: Isolation of the Pathogen: Detection of the Virus Cytopathic Effect (ECP) on Cell in Culture in Vitro

Peste des Petits Ruminants Virus (PPRV)  
Rinderpest Virus (RPV)
Drawbacks of those tests:

- Sometimes very difficult to implement
- In many cases: Too long to obtain results
- Poor growth of pathogen in vitro
- Not enough sensitive
- Cross reactions with other pathogen (lack of specificity)
- Impossibility to handle many samples at the same time

Finally less efficient for quick control of infectious diseases, particularly those of Transboundary nature
For Efficient Control of these Diseases, the Needs for Diagnostics Tests, Key Topics, are:

- Rapid Tests
- Highly Sensitive tests
- Highly Specific tests
- Tools to Enable Tracing back the evolution of the Disease and also to see evolution of the pathogen
- Possibility to handle high number of samples at the same time (High throughput)
- Possibility to carry out the test on the site (Penside test)

Tests should not be too expensive (Important criteria for the developing world)
ELISA: Dramatic Improvement in Serological Tests
Nucleic Acid-Based pathogen detection: DNA Probes

RPV and PPRV Detection by radioactive Probes

PPRV Detection by non-radioactive Probes
Classical PCR

Detection of SPPV in Pathological Samples
REALTIME-PCR TECHNOLOGY

Advantages:
- Post PCR of product analysis on gel no more needed
- Cross contamination dramatically reduced
- Rapid
- More sensitive than non-nested classical
- Quantification of the material
Detection of PPRV by TaqMan MGB real time PCR

The amplification plots were realized on 10-fold serial dilutions ($10^7$ to $10^1$ copies) assayed in triplicate using a plasmid containing the PPRV N gene.
Possibility of Genotyping:

CaPVs Genotyping by FRET

The FMCA allows distinguishing individual isolates belonging to the SPPV, GTPV and LSDV groups.

- **SPPV**: Tm = 49 °C, 5 mismatches
- **LSDV**: Tm = 58 °C, 3 mismatches
- **GTPV**: Tm = 65 °C, 0 mismatch
GeneSTAT® - Portable PCR

Analyzer

Fully automated system for rapid DNA & RNA analyses

Sample preparation included

Simple one touch operation

Light weight and field deployable

Test Module

Pocket-sized, sealed cartridge (biohazard friendly)

Months to years storability at ambient temperatures
Isothermal Nucleic Acid Amplification: Loop – mediated Isothermal Amplification (LAMP)

Detection of the amplified products by electrophoresis on gel

OR

Simply under UV light after having added to the solution a ds DNA binding fluorophore

Use of at least 4 primers to amplify DNA by a DNA polymerase with strand displacement activity (Bst1), amplification carried out at a single temperature
PENSIDE Test: IMMUNOCROMTOGRAPHY TEST for early detection, to control the spreading of infection

No line in the "T" position indicates a NEGATIVE test result.

A line in the "C" position indicates a valid test.

A line in the "T" position indicates a POSITIVE test result.
Data collection, collation and visualisation framework using EpiCollect and www.spatialepidemiology.ne
Improvement of Diagnostic Tests

More Information on Pathogen Genome Sequence

Design of Tests
More Specific

Tracing the Disease Spread and Study of the Pathogen Evolution (Mutants)

Pathogenicity Study with Improvement of vaccine
Pathogen Full Genome Sequencing

For the past 30 years, the Sanger method has been the dominant approach and gold standard for DNA sequencing. The commercial launch of the first massively parallel pyrosequencing, 454 DNA sequencer platform, in 2005 ushered in the new era of high-throughput genomic analysis now referred to as next-generation sequencing (NGS).
# Commercially Available Next-Generation Sequencing Platforms

<table>
<thead>
<tr>
<th>Sequencing system</th>
<th>Feature generation</th>
<th>Sequencing by synthesis</th>
<th>Estimated system cost</th>
<th>Consumable cost per single-end run (paired-end)</th>
<th>Read length per single-end run (paired-end)</th>
<th>Gigabases sequenced per single-end run (paired-end)</th>
<th>Run time per single-end run (paired-end)</th>
<th>Read accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 Genome sequencer FLX</td>
<td>Emulsion PCR</td>
<td>Polymerase (pyrosequencing)</td>
<td>$500,000</td>
<td>n/a</td>
<td>250-300 bp (2X110 p)</td>
<td>0.1 Gb (0.1 Gb)</td>
<td>7.5 hours (7.5 hours)</td>
<td>99.5%</td>
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<tr>
<td>Illumina Genome Analyser</td>
<td>Bridge PCR</td>
<td>Polymerase (reversible terminators)</td>
<td>$400,000</td>
<td>$3000 (n/a)</td>
<td>36 bp (2X36 bp)</td>
<td>1.5 Gb (3.0 Gb)</td>
<td>2.5 hours (5 hours)</td>
<td>&gt;98.5%</td>
</tr>
<tr>
<td>ABI SOLiD System</td>
<td>Emulsion PCR</td>
<td>Ligase (Octamers with to-base encoding)</td>
<td>$525,000</td>
<td>$3390 ($4390)</td>
<td>35 bp (2X25 bp)</td>
<td>3.0 Gb (4.0 Gb)</td>
<td>5-7 days (10 days)</td>
<td>99.94%</td>
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<tr>
<td>Helicos Heliscope</td>
<td>Single molecule</td>
<td>Polymerase (asynchronous extensions)</td>
<td>$1,350,000</td>
<td>n/a</td>
<td>25-35 bp</td>
<td>7.5-10 Gb</td>
<td>3-7 days</td>
<td>&gt;99%</td>
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</tbody>
</table>
The Genus *Capripoxvirus* (CaPV): *sheep poxvirus* (SPPV), *goat poxvirus* (GTPV) and *lumpy skin disease virus* (LSDV)

Pox-like lesions appear on the skin, and various organs

Nomenclature based on the host of origin
Some reports indicate the involvement of both sheep and goats in some outbreaks

*Infections cannot be distinguished clinically or serologically*
The full genome phylogeny shows that CaPVs are more divergent than Thought.

Two subgroups were found within the GTPV group.

Some strains are outside the group corresponding to the host from where they have been isolated.

The strain designation should not rely on the host from where the virus was first isolated.

From the genomic data and the pathogenicity, it appears that some CaPV strains have a complex behavior.
The full genome sequencing of the Marocco vaccine strain has revealed the existence of 7 other potential genes for attenuation.

<table>
<thead>
<tr>
<th>SPPV A</th>
<th>GB</th>
<th>TU</th>
<th>Iliskhi</th>
<th>Djelfa</th>
<th>Denizli</th>
<th>Corum</th>
<th>Mar</th>
<th>GTPV SA</th>
<th>Ortholog Group Name</th>
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<tbody>
<tr>
<td>SPPV-A-002</td>
<td>2</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
<td>27.9</td>
<td>Virulence factor (Cop-B9R)</td>
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<tr>
<td>SPPV-A-003</td>
<td>3</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>24</td>
<td>IL-10</td>
</tr>
<tr>
<td>SPPV-A-004</td>
<td>4</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>49</td>
<td>IL-1 receptor (LSDV-N-006)</td>
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<tr>
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<td>7</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>54.7</td>
<td>Alpha-amanitin sensitivity</td>
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<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>Ribonucleotide Reductase small subunit</td>
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<td>79</td>
<td>x</td>
<td>x</td>
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<td></td>
<td></td>
<td></td>
<td>x</td>
<td>NTPase, DNA replication</td>
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<td>83</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>mutT motif/NPH-PPH/RNA levels regulator</td>
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<td>91</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>Core protein (Cop-A4L)</td>
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<td>96</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>Membrane protein (Cop-A9L)</td>
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<tr>
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<td>113</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>IMV MP/Virus entry (Cop-A28L)</td>
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<tr>
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<td>124</td>
<td>x</td>
<td>x</td>
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<td></td>
<td></td>
<td>x</td>
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<td></td>
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<td></td>
<td>x</td>
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<td>x</td>
<td></td>
<td>56.8</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
<td>Ankyrin (SPV-N-144)</td>
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<td>SPPV-A-149</td>
<td>146</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>27.9</td>
<td>Virulence factor (Cop-B9R)</td>
</tr>
</tbody>
</table>

8 genes in SPPV are likely to affect the virulence because there are highly disrupted in vaccine strains but well conserved in virulent field strains.
Diagnosis of MULTIPLE Diseases in ONE STEP: MICROARRAY
New Generation DNA sequencing technologies

Metagenomics

Possibility to quickly Identify Unknown Pathogen (emerging Pathogens)
Pathogen very difficult to Cultivate in Vitro
Minor Part of Mixed Pathogens Infection
Viral particles are separated from host contaminants using centrifugation and filtration. Viral particles are treated with DNAse I to remove contaminated nucleic acids.

Random priming is used to generate 500–1000 bp amplicons which are size-selected, cloned and sequenced.
New Generation DNA sequencing technologies

PCR, Realtime PCR, LAMP, Microarray, Luminex, Metagenomics, MLPA, Padlock Probes, Biosensor, etc…

RISK of Loosing the GOLD STANDARD Technique for DISEASE DIAGNOSIS: PATHOGEN ISOLATION

NEED to Combine New Technologies with Historical Tests: Isolation of Pathogens
Improving PPRV in vitro Isolation: Development of CV1 Cell line expressing the virus receptor, the Sheep SLAM

CHS cells (CV1 cells expressing the PPRV receptor: SLAM protein):
- Photo A: Syncytia indicated by the arrows in the cell layer infected with PPR suspected pathological sample. This virus cytopathic effect (cpe) appears 2 days after infection instead of 2-3 weeks for normal cells
- Photo B: Control cell, no syncytium detected.
Progress Made in Improving Disease Diagnostic Test

Is a Combination of Progress Made in Many Disciplines

- Molecular Biology
- Immunology
- Chemistry
- Physics
- Informatics
THANK YOU VERY MUCH FOR YOUR ATTENTION