Review on Pen-Side Tests: Prospects in Animal Disease Diagnosis and Eradication Program

Abdi Feyisa, Bethel Befekadu

Abstract-Infectious animal diseases have a great economic and public health impact in developed and developing countries of the world. Thus, early detection for early intervention as well as strategic prevention and control for successful eradication needs rapid and efficient diagnosis tests. Thus, this paper reviews the available pen-side tests and their features in veterinary disease diagnosis and eradication programs. Human and veterinary doctors use diagnosis that ranges from physical diagnosis to sophisticated molecular techniques to reach a reliable conclusion. However, these diagnostic techniques either conventional or modern have limitations in terms of time, cost, availability, portability, ease of use, and effectiveness. Nowadays, different pathogen or disease-specific pen-side tests have been developing to overcome such limitations. Pen-side tests are on-the-spot diagnostic tests with good sensitivity and specificity, ease of use, and affordability by resource-poor countries. California mastitis test and rose Bengal tests are pen-side tests that have been used in veterinary medicine for a long period for early detection and prevention of mastitis and brucellosis, respectively. Today, the lateral flow assay test is becoming the most common pen-side test developing and improving for fast animal and human disease diagnosis. Lateral flow assay for foot and mouth disease and lateral flow assay for Peste des petits ruminants are some examples of lateral flow assay that have veterinary uses. Similarly, loop-mediated isothermal amplification and mobile polymerase chain reaction are among the recently developed point-of-care tests but have limited trials in veterinary medicine. Therefore, pen-side tests are promising diagnostic tests for the future of on-the-spot animal disease diagnosis and eradication programs.

Keywords- Diagnosis, Eradication, Pen-side tests

1. INTRODUCTION

Infectious diseases are caused by infectious organisms such as bacteria, viruses, fungi and helminthes which are a threat to human and animal health. These infectious diseases have a detrimental impact on animal production and productivity hence, the livelihood and the national economy. For instance, some infectious diseases cause reproductive problems by causing infertility or abortion and some may cause extensive death to young and adult animals. Other diseases may cause heavy losses in productivity this can be expressed by loss of quality and quantity food, by products or work performance. Zoonotic diseases are also another problem caused by infectious diseases. These problems are worrisome especially to resource-poor developing countries [1]–[3]. Thus, early detection and prompt intervention measures are required to alleviate the impacts of the diseases on animals, livelihoods, and the national economy. Diagnosis is a center of medicine. Adequate and prompt treatment of a disease can't be made properly without diagnosis. Sensitive, specific and rapid diagnostic testing is not only for the treatment purpose but also plays a critical role in preventing the transmission of infectious diseases [4].

Diagnosis of infectious disease contains identification of an infectious agent either directly or indirectly. It is nearly always initiated by medical history and physical examination. However, many diseases cannot be clinically distinguished from those of other infectious diseases. Therefore, they require definite laboratory confirmation [5], [6]. Each diagnosis step has its own advantages and disadvantages [7]. For instance: traditional laboratory diagnoses are performed by culture, staining and phenotypic characteristics such as fermentation profiles of bacteria, antibiotic susceptibility testing, cytopathic effects in the tissue culture for viral agents, ova and parasite microscopy examination, and antigen detection via immunoassays. These techniques are reliable but time-consuming and their sensitivity can vary. Additionally, such analyses can be easily performed when trained personnel is available [8]–[10].

Nowadays, many other diagnostic methods were developed to identify the underlying pathogen in infectious diseases [11]. The molecular techniques are the one that increasingly being used in clinical diagnosis, treatment monitoring and epidemiological studies. They are slowly replacing the conventional methods [10]. However, most of the modern diagnostic methods require the availability of a steadfast laboratory facility, stable reagents, highly skilled laboratory personnel, multistep sample handling or preparation and adequate provision for logistics support

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which is very important for sample collection and transportation [12].

The problems associated with both conventional and modern diagnosis methods call for simple and cheap pen-side diagnostic kits because such tests can be conducted by vets with minimum instructions, it would save time and resources and also avoid the problems associated with the transportation of samples to the lab [13]. Thus, this review paper provides information on recent developments of pen-side tests and their future prospects in animal disease diagnoses and eradication.

2. PEN-SIDE TESTS

2.1. Definition

Pen- side test has termed in various synonyms as portable test, field test, on side test, point of care test etc. [14]. College of American Pathologists recently gives a more restrictive and comprehensive definition of point of care testing, it said "Point of care testing is analytical patient testing activities provided within the institution, but performed outside the physical facilities of the clinical laboratories. It does not require permanent dedicated space, but instead includes kits and instruments, which are either hand carried or transported to the vicinity of the patient for immediate testing at that site" but this definition lacks remote use of the test [15].

According to WHO, point of care tests that address infectious disease control programs, especially for the developing countries, should follow "ASSURED" criteria that are affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users [4].

2.2 History of Pen-Side Tests

The idea of body fluids rapid diagnostic tests is dates back to ancient history. One of the earliest records of a urine-based pregnancy diagnosis can be found in ancient Egypt. In this test, a potentially pregnant woman could urinate for several days on wheat and barley seeds. The result of this test: "If she's not pregnant neither of two grows; If it is a boy, barley grows; If it is a girl, wheat grows" Interest in urine as a rapid diagnostic medium continued through the middle ages by seeing the color of urine. Healers in 1500 BC observed that ants were drawn to the patient's urine that had a mysterious emaciating disease. In the 1600s, tasting the urine was the method to diagnose diabetes. But the earliest and most basic point of c are test was dipstick urinalysis [16].

For infectious diseases first large-scale use of the immunoassay was reported by Dochez and Avery in 1917 in case of detection of pneumococcal polysaccharide from pneumonia patient serum and urine. The authors in that prescient comment suggested that antigen detection could enable a rapid diagnosis of infection [17].

2.3 Features of Pen-Side Tests

While central clinical laboratory tests such as blood culture, high-throughput immunoassays, polymerase chain reaction (PCR) and mass spectrometry (MS) tests offer sensitive and specific assays, they are often time consuming and needs intensive labor, costly and dependent on sophisticated instruments and well trained operators. On the other hand, point-of-care (POC) tests deliver a rapid on-spot results at the site of care delivery and in resource-limited settings, support time management and proper treatment [4]. They also play an important role in resource-limited countries [17].

Rapid antigen-based diagnostic tests to some of the infectious diseases are being manufactured in small quantities by the designers and being used locally. International firms and their local distributors are not interested to provide this tests for developing countries because they believe they will not be profitable [18]. If there is lack of proper management the device can lead to false result [19].

3. TYPES OF PEN-SIDE TESTS AND THEIR APPLICATION IN VETERINARY MEDICINE

Pen-side diagnostic testing is often vital to obtain and offer real time status of an animal or herd health status. It has an application in a disease outbreak, where the clinician is presented with dead or dying animals, in this case rapid results are obtained by using the pen-side test. Second application of this test is as a part of preventive medicine/herd health program. Because information found from the test can be used for determining herd incidence or prevalence. Another application of the test is as a part of health certification before sale or stocking, or during processing following slaughter [20].

3.1. Lateral flow assay (LFA)

Lateral flow assays continue to be widely used in biology and medicine for the detection of small quantities of an antibody or antigen of interest in fluid test samples. LFA tests are designed for use at a point of care and they are significant for diagnostic purposes of infectious diseases. They have become very popular during the last decade. LFAs are work based on the principle of immunochromatography. Thus immunochromatographic strip test is the other name of this test. Lateral flow assays are prefabricated strips of a carrier material containing dry reagents that are activated by applying the fluid sample, are important for diagnostic purposes [21]–[23].

The format in LFT is similar to ELISA. ELISA is used for the detection and quantification of specific antigens or antibodies in a given sample. It uses the basic immunology concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample. The base substrate is nitrocellulose membrane in which immobilized capture binding protein. Labels such as latex, colloidal gold, carbon, and recently up-converting phosphorus technology have been employed in LFT development [24], [25].

Lateral Flow Test principles and assay format

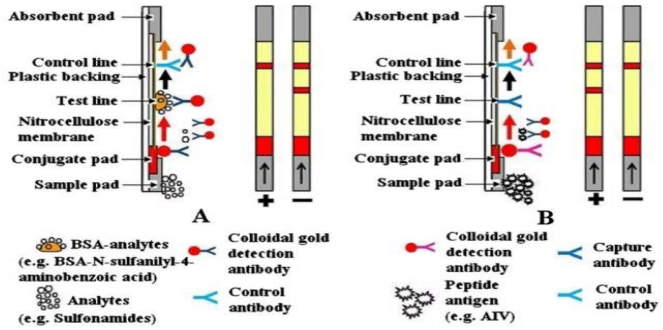
There are various possible formats depending on the type of target analyte. The two kinds of format frequently used are sandwich assay and competitive assay. Multiplex detection format is also recently widely used format [26], [27].

Sandwich format

Sandwich assay format is used to test analytes that have several epitopes such as viral protein. This assay can employ two different antibodies; polyclonal and monoclonal. These antibodies bound distinct epitopes of the analysts: a labeled polyclonal antibody serve as detector reagent and placed in conjugate pad where as a monoclonal antibody (specific to the analyte) is serve as capture reagent and sprayed at the test line of the nitrocellulose membrane. There is also a control line used to check whether the test is working or not. When sample is applied on the conjugate pad the analysts in the sample bind with the polyclonal labeled antibody. Because these antibodies are temporarily adhering when the analyst containing fluid come it will be washed and go to the test pad then then analyte will be sandwich between two antibodies. The response in the capture zone (test line) is directly proportional to the amount of analyte in the sample [24], [28].

Competitive format

Competitive format is best for low molecular weight compounds or which have a single epitope. Competitive format has two layouts. In the first layout, solution containing target analyte is applied onto the sample application pad and prefixed labeled biomolecule (antibody/aptamer) conjugate gets hydrated and starts flowing with moving liquid. Test line contains pre-immobilized antigen which binds specifically to label conjugate or if the sample contain the target analyte it will bind to it. So here the analyte in the sample and the labeled antibody compete for the antigen. Test line contains pre-immobilized antigen that specifically binds to label conjugate. Here if target analyte in sample solution is absent or present in low amount, pre immobilized antigen will bind to the labeled conjugate. If the target analye is present it will bind to the antigen and no signal will found. This means absence of color at test line is an indication for the presence of analyte whereas presence of color both at test and control lines indicates a negative result [21], [27].



A: Competitive LFT format B: Sandwich LFT format

Fig1. **Formats of lateral flow assay tests, Source:** [24]

Multiplex detection format

Multiplex detection format is used to analyze multiple analytes simultaneously under same set of conditions. Therefor more than one target species and assay is done over the strip containing test lines that are equal to number of target species to be analyzed. There are different strategies for multiplexing lateral flow immune assay. Those are: integrating several analytes test lines or dots together this is the most popular strategy for multiplexing LFIAs, by collecting several individual strips and arranged in a special holder and by conjugating probe between a recognition elements [22].

Lateral flow assay application in significant veterinary diseases

Lateral flow assay for Foot and mouth disease

Accurate and rapid confirmatory diagnosis of FMD is necessary to recruit control strategies and trace the source of an outbreak. There are routine diagnosis techniques of FMD which are used in combination. Those are enzyme-linked immunosorbent assay (ELISA), virus isolation techniques and reverse transcriptase PCR (RT-PCR). However, most of these diagnostic approaches require management of the logistical considerations associated with sample collection and transportation. And other fundamental requirements like dedicated laboratory facility, multistep sample handling or preparation, highly trained laboratory personnel and stable reagents [12]

To avoid such problems a need for rapid and easy to perform test, which would allow for on-site diagnosis to be

made in the case of a suspected disease outbreak is useful in areas where the disease is endemic. There for pen-side diagnostic tests would have the advantage of rapid, approachable, correct identification of a particular strain and economically feasible diagnosis of FMD in field condition [29].

A rapid chromatographic strip test for the pen-side detection of FMD virus antigen was developed by Reid *et al* and it has a very significant result in which the test system was more sensitive than ELISA for the diagnosis of all seven serotypes of FMD virus in the nasal swabs and epithelial suspensions and had equivalent sensitivity to the ELISA for the detection of contemporary virus strains in cell culture supernatant fluids. This pen-side test also found rapid and less costly [30].

Study carried by Oem *et.al* also reveals that four FMDV serotypes (type O, A, Asia 1, and C) were detected in the lateral flow assay. The diagnostic sensitivity of the LFA for FMDV types O, A, C, and Asia 1 was approximately 87.3% which is similar to 87.7% by obtained ELISA. The diagnostic specificity of the LFA was 98.8%, compared to 100% for the Ag-ELISA [31].

Another test developed for FMD by Saxena *et.al* was a multiplex agglutination form. It is cheap and simple prototype pen side kit used to differentiate serum antibodies against different serotypes of Foot and Mouth Disease (FMD) virus. The result confirmed that this pen-side test is more sensitive and minimizing false negative results [13].

Lateral flow assay for Rift valley disease .

As RVFV presents a high biohazard for the livestock as well as for human, to do conventional serological tests and molecular tests biosafety level 3 laboratories is required but often that are not available in the areas where the disease is prevalent. Conventional techniques for the diagnosis of RVF include virus isolation, detection of specific IgG or IgM antibodies and detection of specific nucleic acids. Therefor ELISA is validated for the serological test of RVF. Conventional and real-time reverse transcriptase polymerase chain reaction assays are currently the most rapid and sensitive tests for the detection and quantification of RVFV during outbreaks. Other methods like high-throughput **sequencing** are also used. But most of these techniques are expensive and require dedicated trained personnel [32]–[34].

A novel lateral flow strip test for RVFV has recently been developed by Cêtre-Sossah *et al.* for use as a pen side diagnostic test. This test records a high level of diagnostic accuracy with almost 100 percent sensitivity and 98.8 percent specificity, with the absence of cross-reactivity with viruses belonging to different genera. As the researchers indicate lateral flow immunochromatographic strip test is become very valuable first-line diagnostic tool for RVF disease in resource-poor diagnostic territories for on-site RVFV detection because no specialized reagents and laboratory equipment are required [34].

Lateral flow assay for brucellosis

Because brucellosis has no pathognomonic sign it may confuse with other diseases there for laboratory confirmatory tests are mandatory. Among these tests, only the isolation of the microorganism provides absolute proof of infection but bacteriological diagnosis is expensive and dangerous. Even though serological tests like ELISA have great aid in the diagnosis, they needs well equipped laboratories and/or adequate budgets, they cannot be implemented in many laboratories in endemic areas [35].

Lateral flow assay is also other point of care test in brucellosis that gets an attention nowadays. In the evaluation of LFA as a field test for investigation of brucellosis outbreak in an organized buffalo farm, showed 87.1% sensitivity and 92.6% specificity of the test, which was even higher than the specificity of RBPT. In another evaluation of LFA for human brucellosis confirm the high sensitivity of the assays. There for these studies confirm that LFA is a useful, rapid and easy-to-perform tool in the diagnostic testing of brucellosis [36], [37].

Chromatographic strip test for Rinderpest

As OIE a vital step towards eradication of rinderpest as part of global eradication program was the cessation of vaccination and shift to active surveillance of regions declared free of rinderpest. This active surveillance was supported by a rapid diagnostic test, which provided diagnosis at pen-side [38].

A prototype rapid chromatographic strip pen-side test was developed for the detection of rinderpest virus (RPV) antigen in lachrymal fluid of cattle is described by Bru¨ning *et.al*. This test shows high sensitivity which is even more sensitive than immunocapture ELISA. Generally the study confirms that chromatographic strip test strip for RP disease has several advantages over other tests. Like its rapidity (5–20 min), smart in field application, easy to handle and does not require a large amount of sample (100 ml). While it is not as sensitive as the laboratory-based RT-PCR [39].

Another improved rapid chromatographic strip-test for the diagnosis of rinderpest and peste des petits ruminants

(PPR) viruses was development by the same researchers. For rinderpest the test incorporate monoclonal antibody (Mab), which can recognizes additional strains of RPV. The test had high accuracy as the previous one [38].

Chromatographic strip test for Peste des petits ruminants (PPR)
Laboratory verification is an absolutely necessary because a rinderpest is also a viral disease which affect sheep and goat and differentiate between them is impossible unless the presence of confirmatory diagnosis. Laboratory diagnosis of PPR includes virus isolation, detection of specific antibody in the serum, detection of viral antigens, nucleic acid isolation and sequencing [40].

But as a FAO recommendation pen-side diagnostic test for the detection of PPRV in goats and sheep would be beneficial to any control/eradication program for PPR and become a next focus area. A rapid chromatographic strip pen-side test which was developed for PPR and rinderpest was recognize a wide range of PPRV isolates and did not show any cross-reactivity with any other tested viruses therefor it will be helpful for future PPR control eradication programs [38].

Chromatographic strip test for Rabies
The diagnosis of rabies is made by isolation of the virus from tissue or secretions, detection of viral antigen in tissue samples (brain, corneal smears or rarely CSF), serologic evidence of acute infection or molecular techniques such as PCR or genetic probes [41]. As OIE recommendation rapid and accurate diagnosis of rabies is important for establishing rapid control measures and for advising the exposed people for post exposure treatment [42].

In the evaluation of a rapid immunochromatographic test for the diagnosis of rabies by Tenzin *et.al* a very successful result was found. The test indicates sensitivity of 92% and specificity of 100%. The study conclude that rapid test kit can be used for rabies surveillance and for confirmation of clinical case of rabies in animals for making rapid decisions particularly in controlling rabies outbreaks in resource poor areas [43].

Chromatographic strip test for Canine parvovirus
Diagnosis of canine parvovirus is very significant to isolate infected dogs and prevent transmission to susceptible animals. Since several other pathogenic organisms can cause diarrhea in dogs, diagnosis on the basis of clinical signs is not definitive. Therefore, a clinical diagnosis should always be confirmed with laboratory tests. Various laboratory methods have been developed to detect canine parvovirus in the. They include electron microscopy (EM), ELISA, haemagglutination (HA) tests, viral isolation (VI), haemagglutination inhibition (HI) tests, conventional polymerase chain reaction (C-PCR) and real-time polymerase chain reaction (RTPCR) especially in kennels and shelters in order [44].

The rapid immunochromatographic test here was developed for the detection of canine parvovirus (CPV) in dog feces. In addition also it can be used for the diagnosis of infections with viruses causing parvovirus enteritis in cats and mink. The result of the evaluation showed an overall relative sensitivity and specificity of 95.8 and 99.7%, respectively. These results show that this one-step test is simple, rapid, reproducible and sensitive diagnostic test for the detection of parvovirus in faecal samples of dogs, cats and mink [45].

3.2. Loop-mediated Isothermal amplification (LAMP)

After the development of polymerase chain reaction gene amplification method has been used for widespread clinical use, particularly in genetic testing. The steps of genetic testing include nucleic acid extraction, gene amplification and detection. But these steps need considerable skill and expensive equipment and facilities and making convenient testing at any given location difficult. To overcome these limitations a new gene amplification method called the loop-mediated isothermal amplification (LAMP) developed [46].
Loop-mediated isothermal amplification (LAMP), a newly developed gene amplification method designed and developed by Notomi *et.al* and coworkers. It combines rapidity, simplicity, high specificity and low cost. LAMP amplifies nucleic acid under isothermal conditions, which is highly compatible with point-of-care (POC) analysis. The reasons for the development of the LAMP methodology were founded on the attempt to overcome some drawbacks of the conventional PCR, a method that requires the acquisition of high-cost equipment known as thermal cycler. LAMP does not require any specific equipment [47].

Principles of LAMP
LAMP amplification is carried out without the need of thermal cycler at a constant temperature. This method amplifies very few copies of target DNA with high specificity, efficiency and rapidity. Four different primers are used for identification of six distinct regions on the target gene, which usually increases its specificity and makes it to produce higher DNA than the traditional PCR. Amplification and detection of gene are completed in a single step by incubating the gene sample, DNA polymerase, and substrates at constant temperature. Gene amplification in LAMP is done through repetition of template self-elongation from the stem loop structure and subsequent binding and elongation of new primers to the

loop region. In this reaction, pairs of inner and outer primers are used. The elongation reactions are sequentially repeated by DNA polymerase enzyme [48]. Result reading can be based on visual inspection, ultraviolet fluorescence and agarose gel electrophoresis, etc.[49].

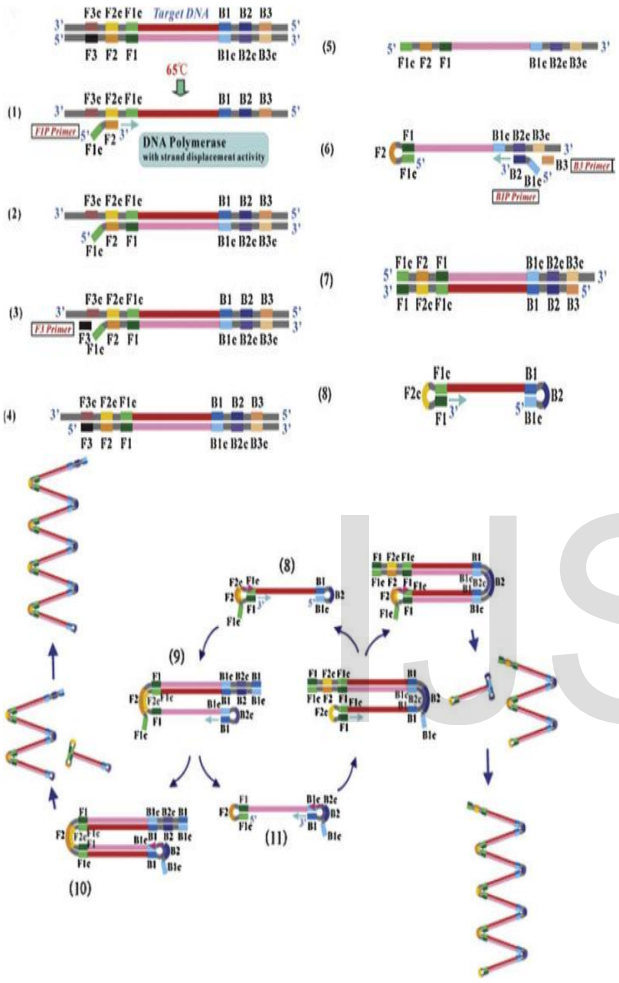


Fig 2. Principle of LAMP; Source: [49]

LAMP in veterinary application

As Eular *et al.* report isothermal nucleic acid amplification methods have gained popularity for their ability to more rapidly detect pathogen nucleic acids directly in a clinical or field sample and can be used in the diagnosis of Rift valley fever virus, Japanese encephalitis virus and African swine fever virus but compared to qRT-PCR they are less sensitivity. Their simplicity portability of this system could be a potential tool for field, pen side or bedside diagnostics [50].

As Europe food safety authority on the study of lumpy skin disease report different researchers have been published study on Loop-mediated isothermal amplification assays (LAMP) but they are not yet ready for pen-side use. The performance of the test in the report of EFSA ranges from 70 to 100 % for the sensitivity and from 92.3 to 100 % for specificity. But suitable pen-side extraction method for the LAMP assay still needs to be developed [51].

3.3. Mobile pen-side PCR

Mobile pen-side PCR was recently developed, and it has promising results. It is easy to use in field conditions and results are obtained within one hour. Owing to the freeze-dried reagents, no cold-chain is needed in the field setting, sample collection is easy and no separate DNA extraction is required. In this research it is reported that, this pen-side PCR is routinely used for CaPV at the OIE reference laboratory in Pirbright. A pen-side test will provide rapid confirmation for the tentative field diagnosis allowing a swift implementation of stamping-out and movement restrictions and therefore enhancing the efficacy of the eradication and control measures [51].

As a report of Boehme *et al.* recently a single use closed PCR system has been developed, for the diagnosis of *Mycobacterium tuberculosis* complex infections in humans and the identification of antimicrobial multi-resistance using sputum samples but has no veterinary validation. This test allows for significant automation with all reagents used for bacterial disruption, nucleic acid extraction, amplification and amplicon detection inside a disposable container. Results are generated within 2hr of processing and to be promising. Test sensitivity evaluation recorded 98.2% in smear positive patients and 72.5% in smear negative patients [52].

3.4. Other pen-side tests

California mastitis test (CMT)

A number of screening tests, each with their own strengths and weaknesses are available to aid in the diagnosis of mastitis including direct measurement of the somatic cell count (SCC) level, California Mastitis Test (CMT) on suspected quarters, milk culture of suspected quarters or cows or composite, Bulk tank somatic cell counts (BTSCC). The CMT has been used for more than 50-years and continues to be the most accurate cow-side screening test for subclinical mastitis [53], [54]. The CMT is a quick, inexpensive, simple cow-side mastitis test that accurately forecasts the somatic cell count of milk from individual quarters or on composite milk samples. It estimates the number of somatic cells present in milk. Somatic cells are composed of approximately 75% of leucocytes and 25 % of epithelial cells (secretary and lining cells). As Leucocytes

are body's primary defense cell, there increase in mastitis is enviable. However, rise in epithelial cells is due to an injury to mammary epithelium [55], [56].

CMT test principle is when CMT reagent mix with an equal amount of milk it directly the CMT reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of leucocyte, which are primarily compose of fat and then DNA is released from the nuclei. DNA will form gel or stringy mass together. As the number of leucocytes in a quarter increases, the amount of gel formation will increase. Therefore, the gel formation will have score now as 0 (If no SCC), T (Trace), 1, 2 and 3 (if high amount of SCC is there) [57].

Figure 2: California milk test result in increasing order; Source: [56]

Assessment of sensitivity and specificity of CMT is done in different studies. Most of the tests show that CMT has good sensitivity than other screening techniques. In the study of Badiuzzaman *et.al* the sensitivity of CMT is 80.08% and 69.40% of specificity. This result is more than other screening tests except somatic cell count. The same result was found by Hoque et.al and Cole et.al [58]–[60].

Nowadays, lateral immuno flow assay is used for the diagnosis of mastitis. A semi quantitative lateral flow assay for the detection of SCM in dairy cows targeting myeloperoxidase (MPO) enzyme of milk neutrophils was developed by Alhussien and Dang. They found this test has a greater accuracy than 97% [61].

Rose Bengal plate agglutination test (RBPT)

The RBPT is a simple, rapid, spot test and can be performed in field. Rose bengal has been used for about 140 years but not principally as a therapeutic agent. This test is internationally acknowledged as the choice for the screening of brucellosis. The test depends on the ability of antibody in the patient's serum to agglutinate the stained bacterial antigen. When the test is positive the agglutination becomes clearly visible to the naked eye. The low pH of antigen prevents some agglutination by IgM and encourages agglutination by IgG thereby reducing non-specific interactions [62]. Even though the low pH of the antigen is good for specificity of the test, the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity [63].

The study on comparative assessment of sensitivity and specificity of RBT and modified in house ELISA for the diagnosis of brucellosis shows the effectiveness of RBT in the diagnosis of Brucellosis because it is more sensitive and accurate test than ELISA as well as cost effective [64].

In other systematic review the performance of RBT was evaluated. In this review the diagnostic sensitivity (DSe) of RBT was found 87.5% and the specificity (DSp) was found100%. The conclusion of this review shows the good diagnostic performance of RBT combined with its rapidity, simplicity, and affordability makes RBT an ideal ne point-of-care test for early clinical diagnosis and management of brucellosis [65].

4. PEN-SIDE TESTS IN CONTROL AND ERADICATION

Diseases control and eradication are reduction of specific disease prevalence to relatively low level and to the point of continued absence of transmission and occurrence respectively. These programs bring tangible profits like in promoting animal health and welfare issues, and reduced direct and indirect farm production losses. An essential component of any disease control strategy includes the development of diagnostic assays [31], [66].

To make disease controlling strategies effective, the diagnosis should be at early stage and effective. For instance, in case of disease outbreak, the disease should detect early and persistent infections should also be recognized to prevent further transmittance. These can be

achieved when available diagnostic tools are specific and sensitive and rapid [12].
The steps in the outbreak are when the earlier the clinical signs of disease are recognized by the farmer and the earlier the clinical diagnosis is confirmed by laboratory tests, along with prompt reporting to the relevant veterinary authorities, then better disease controlling will be established. The availability of pen-side diagnostic tests could achieve these goals because the time needed to confirm the disease is short and tests can be conducted in the field, at the site of the outbreaks [34]. For example; a pen-side diagnostic test for control and eradication program for PPR, given the success of GREP and FAO looks at it as the next targets point for control or eradication of PPR [14], [38].

Another example is a study in lumpy skin disease which reach in a conclusion that rapid laboratory confirmation of suspected LSD field cases is essential for successful eradication of the disease this can be achieved by pen-side test which allows swift implementation of stamping-out and movement restrictions and therefore enhancing the efficacy of the eradication and control measures [51].

5. CONCLUSIONS

Pen-side or points-of-care tests are prompt, on-the-spot, affordable and easily used diagnostic tests in human and animal diseases diagnosis. Different pen-side tests have been developed and developing for the animal disease diagnosis. The advancement and proper implementation of these diagnostic tests will assist clinicians to easily reach at a definitive diagnosis. This contributes directly or indirectly to animal disease control and eradication program, further the public health and the national economy. Thus, the pen-side tests are promising diagnostic tests for the feature of animal diseases diagnosis and eradication program.

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