ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA LOMBARDIA E DELL' EMILIA ROMAGNA
"BRUNO UBERTINI"

SELEZIONE PUBBLICA PER TITOLI E COLLOQUIO PER EVENTUALI ASSUNZIONI A TEMPO DETERMINATO DI PERSONALE NEL PROFILO DI COLLABORATORE TECNICO PROFESSIONALE ADDETTO AI SERVIZI DI LABORATORIO CAT. D CON COMPETENZE IN SCIENZE ZOOTECNICHE E TECNOLOGIE DELLE PRODUZIONI ANIMALI DA ASSEGNARE ALLE SEDI DELLA LOMBARDIA E DELL'EMILIA ROMAGNA DELL'ISTITUTO

27 giugno 2022 mattina

PROVA ORALE N° 1

Gestione delle contaminazioni in un laboratorio PCR

Add 100 µL of prepared 1X HRP-Streptavidin solution (see Reagent Preparation step 6) to each well. Incubate for 45 minutes at room temperature with shaking. Discard the solution. Repeat the wash as in step 3.

PROVA ORALE N° 3

Circuiti inter-laboratorio: significato e scopo

Remove the tube from the vortex adaptor, add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol to the sample, then shake the tube for 3 minutes at speed 1 or 2 on the vortex adaptor.

PROVA ORALE N° 6

Tecniche di quantificazione della carica virale

Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec). Add 50 µL of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

PROVA ORALE N° 7

Gestione del flusso di campioni e materiali nei laboratori di diagnostica molecolare con PCR

Add 100µl of sterile water, make sure that you cover the lyophilised sera with liquid. Close the lid and leave in room temperature for 15 minutes, mix gently for 10 second with vortex and incubate for 15 more minutes at room temperature before use.

PROVA ORALE N° 8

Caratteristiche di un laboratorio di batteriologia

Add required reagents or mastermix and template to PCR tubes. Mix and centrifuge. Add mineral oil to prevent evaporation in a thermal cycler without a heated lid.

PROVA ORALE N° 9

Tecniche di identificazione batterica

Add the reagents to an appropriately sized tube in the order provided in the table. (Select appropriate table for reaction setup: standard or readymix reagent.) For a large number of reactions, a mastermix without the template should be set up and aliquoted into reaction tubes. At the end, template should be added to appropriate tubes.

PROVA ORALE N° 11

Cappe filtranti. Descrizione delle diverse classi

After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Add 100 µL of 1x prepared Detection Antibody to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

PROVA ORALE N° 13

Principali componenti di un mix di reazione PCR

Analyse PCR products by agarose gel electrophoresis. The products should be readily visible in an ethidium bromide-stained gel under UV light. Reactions containing Green GoTaq® Buffer do not need loading dye added before electrophoresis.

PROVA ORALE N° 14

Diagnosi diretta ed indiretta di una infezione virale

Aspirate wells and wash 1 time with >200 µL of Wash buffer per well. Following wash, invert and tap on absorbent paper to remove excess liquid. Block plate with 200 µL per well with Blocking buffer for 1 hour at room temperature.

PROVA ORALE N° 16

Controlli positivi di estrazione e amplificazione

Biological risk assessments are undertaken to inform and determine the policy and procedures that in turn give confidence that the laboratory procedures for each of the biological materials handled by the laboratory pose negligible danger to a country’s animal and human populations.

PROVA ORALE N° 17

Differenza tra dispositivi di protezione individuale e collettivi

Block the remaining protein-binding sites in the coated wells by adding 200 µL blocking buffer, 5% donkey serum in 1X PBS is used here, per well. Alternative blocking reagents include 5% non-fat dry milk or BSA in PBS or normal serum from an animal in which the secondary antibody was generated.

PROVA ORALE N° 18

Real-time PCR applicata alla diagnostica microbiologica: vantaggi e svantaggi rispetto ai metodi di microbiologia classica

Chemiluminescence detection is recommended when detecting and quantifying low abundant proteins or when sample and primary (capture and detection) antibodies are limited.

PROVA ORALE N° 21

Organizzazione delle aree di prova di un laboratorio di diagnostica molecolare

Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

PROVA ORALE N° 22

Criteri di classificazione degli agenti biologici in base ai rischi di infezione.

Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating. Invert the plate and blot it against clean paper towels

27 giugno 2022 pomeriggio

PROVA ORALE N° 2

Rapporti tra laboratori ad alto contenimento ed ambiente esterno. Presenza di aree dedicate, sistemi di trattamento del materiale in entrata/uscita dai laboratori e dei reflui.

Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Cover wells and incubate for 30 minutes at room temperature in the dark with gentle shaking.

PROVA ORALE N° 3

Prevenzione delle contaminazioni da RNAsi

CRITICAL STEP: All bacterial cultures should be treated as potentially pathogenic to the laboratory worker and colleagues. Therefore, the use of appropriate aseptic techniques, and the wearing of appropriate personal protective equipment are strongly recommended to maintain acceptable work health and safety standards and minimise exposure to harmful agents

PROVA ORALE N° 4

Gestione delle apparecchiature in un laboratorio di prova

Add 100 µL of prepared 1X biotinylated anti-phosphotyrosine antibody to each well. Incubate for 1 hour at room temperature with shaking. Discard the solution. Repeat the wash as in step 3

PROVA ORALE N° 7

Tecniche di isolamento batterico

Discard the solution and wash 4 times as directed in Step 3.Add 100 µL of prepared HRP-Streptavidin solution to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes.

PROVA ORALE N° 8

Controllo interno di una reazione PCR – tipologie

Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel pipette or autowasher.

PROVA ORALE N° 9

Sanger. Basi della tecnica e come si conduce un sequenziamento

Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance.

PROVA ORALE N° 11

Elettroforesi in gel d’agarosio

Gently mix by tapping tube. Briefly centrifuge to settle tube contents. Prepare negative control reaction without template DNA. Prepare positive control reaction with template of known size and appropriate primers.

PROVA ORALE N° 13

Quantificazione mediante Real-Time PCR

Discard the solution. Repeat the wash procedure as in step 3. Add 100 µL of prepared Streptavidin solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.

PROVA ORALE N° 15

Quali controlli prevedere in una PCR

Biological risk analysis is the process of identifying and characterising health, safety, and security risks, followed by implementing, measuring the effectiveness of, and communicating the control measures used to reduce those risks to acceptable levels

PROVA ORALE N° 17

Controlli di processo

Fluorescently labeled probes provide a highly sensitive and specific method of detection, as only the desired PCR product is detected.

However, PCR specificity is also important when using sequence-specific probes

PROVA ORALE N° 18

Sequenziamento importanza e applicazioni

If possible, start with >104 copies of the target sequence to obtain a signal in 25–30 cycles. Excess template is not beneficial to the reaction. Always ensure that the final DNA concentration is ≤10ng/µl. Less than 10 copies of a target can be amplified (2), but more cycles may be required to detect a signal by gel electrophoresis.

PROVA ORALE N° 21

L'Accreditamento dei Laboratori di Prova

For the purpose of laboratory testing to establish a diagnosis it is important to sample animals that are either clinically affected, or suspected on good evidence to be infected or, for serology, to have been infected. Specimens that are most likely to give highest sensitivity and specificity to the investigation should be collected.

28 giugno 2022 mattina

PROVA ORALE N° 1

Vantaggi e criticità dell'impiego di tecniche PCR per la diagnostica

Add 100 µl of each standard, positive control and sample into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4°C.

PROVA ORALE N° 2

Estrazione degli acidi nucleici da colture batteriche

An accredited quality management system (QMS) enables a laboratory to identify, measure, control and improve various core processes that lead to improved performance. In simple terms: - say what you do: document procedures – do what you say: follow documented procedures – improve it: corrective action processes. – prove it: audits and quality control

PROVA ORALE N° 3

Genotipizzazione batterica

Competitive ELISA (C-ELISA) is a variant of ELISA used to detect or quantify antibody or antigen using a competitive method. The principle of a competitive assay for detection of antibodies is competition between antibodies that may be present in the test serum and the detecting antibody (which in this case binds directly to the antigen).

PROVA ORALE N° 4

Caratteristica degli ambienti in un laboratorio di biosicurezza di livello 4

Conventional PCR, does not enable accurate quantification of nucleic acids. Real-time PCR is highly suited for a wide range of applications, such as gene expression analysis, determination of viral load, detection of genetically modified organisms (GMOs), SNP genotyping, and allelic discrimination

PROVA ORALE N° 6

Materiale di riferimento, caratteristiche ed esempi

Discard the solution. Repeat the wash procedure as in step 3. Add 100 µl of prepared Streptavidin solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.

PROVA ORALE N° 7

Principio delle tecniche immunologiche

Each different matrix to be used in an assay must be used in the validation process. Some sample matrices include inhibitory factors that interfere with the performance of specific types of assays.

PROVA ORALE N° 8

Tecniche di semina delle colture batteriche.

Faeces, autolysed tissues and semen samples tend to contain more interfering substances and rare therefore more problematic for assay performance than are serum, blood or fresh tissues.

PROVA ORALE N° 10

Enzyme-linked Immunosorbent assay – ELISA

In multiplex, real-time PCR, several genomic DNA targets are quantified simultaneously in the same reaction. Multiplex, real-time RT-PCR is a similar method, allowing simultaneous quantification of several RNA targets in the same reaction. The procedure can be performed either as two-step RT-PCR or as one step RT-PCR

PROVA ORALE N° 11

Tracciabilità dei reagenti in un laboratorio diagnostico

It is important to minimize cross-contamination between samples and prevent carryover of RNA and DNA from one experiment to the next. Use separate work areas and pipettors for pre- and post-amplification steps. Use positive displacement pipettes or aerosol-resistant tips to reduce cross-contamination during pipetting.

PROVA ORALE N° 14

Descrivere le fasi ed il principio della Reazione a catena della Polimerasi (PCR)

Laboratory investigation of animal disease is critically dependent on the quality and appropriateness of the specimens collected for analysis. This chapter sets out the general standards involved in specimen collection, submission and storage.

PROVA ORALE N° 15

Metodi di rilevazione di un prodotto di PCR/PCR Real Time

Make working solution of Streptavidin-HRP with Blocking buffer by diluting 1:5,000. For example to make enough for 1 plate, add 2 µl of Streptavidin-HRP to 9,998 mL of blocking buffer.

PROVA ORALE N° 16

AGID Immunodiffusione in agar gel

Non-host factors, such as contamination or deterioration of the sample, also potentially affect the ability of the assay to detect the specific targeted analyte in the sample

PROVA ORALE N° 17

Determinazione della carica batterica

Prepare Coating Solution by diluting the Capture antibody in Coating buffer. Refer to manufacturer for dilution recommendations. Coat plates with 100 µl per well of coating Solution. Cover plates, and incubate overnight (12 – 18 hours) a 2 – 8 °C

PROVA ORALE N° 18

Differenza tra tecniche immunologiche dirette e indirette

Prepare sufficient DNA Binding Bead Mix for your sample extraction and store the mix at room temperature. If you are preparing multiple samples, prepare 5% excess volume to account for error.

PROVA ORALE N° 19

Differenza tra sensibilità e specificità diagnostica

Real-time RT-PCR is an ideal tool for cell assays that require accurate analysis of gene expression. However, high-throughput assays are difficult to achieve, since the purification of RNA from large numbers of cultured-cell samples involves both time and effort.

PROVA ORALE N° 21

DPI utilizzati in un laboratorio di biosicurezza di livello 3

Remove the tube from the magnetic stand, add 300 µl of Wash Solution 1 to each sample, then vortex the sample in pulses for 20 to 30 seconds, being careful to prevent beads from sticking to the sides of the tube.

PROVA ORALE N° 22

Campo di applicazione di un metodo di prova

Samples with high amounts of nucleic acid, such as tissue, avian blood and bacterial cultures, can overwhelm the magnetic beads. Overwhelming the beads reduces nucleic acid extraction efficiency.

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PROVA ORALE N° 2

Tecniche di identificazione batterica

Add the reagents to an appropriately sized tube in the order provided in the table. (Select appropriate table for reaction setup: standard or readymix reagent.) For a large number of reactions, a mastermix without the template should be set up and aliquoted into reaction tubes. At the end, template should be added to appropriate tubes.

PROVA ORALE N° 8

Immunofluorescenza diretta e indiretta

To anneal the oligonucleotide primers, the temperature of the next step in the cycle is reduced to approximately 42 – 65°C. At this temperature, the oligonucleotide primers can anneal to the ssDNA strands and serve as primers for DNA synthesis by the polymerase.

PROVA ORALE N° 9

Sybr Green real-time PCR: principi e limiti rispetto ad un saggio TaqMan

Successful amplification of the region of interest depends upon the amount and quality of the template DNA. Reagents commonly used to purify nuclidic acids (e.g. salts, guanidine, proteases, organic solvents and SDS) are potent inhibitors of DNA polymerases.

PROVA ORALE N° 10
Differenza tra ripetibilità e riproducibilità di un saggio diagnostico

Use of oligo-dT primers or random oligomers for reverse transcription means that several different transcripts can be analysed by PCR from a single RT reaction. In addition, precious RNA samples can be immediately transcribed into more stable cDNA for later use and long term storage.

PROVA ORALE N° 11

Confronto PCR, PCR Real Time e PCR nested in termini di sensibilità e specificità

Veterinary diagnostic centres routinely receive specimens that have been submitted because they are suspect for any of a variety of diseases. While the infectious nature of the specimens is unknown, diagnostic case materials may contain a variety of unknown agents, some of which could be extremely hazardous to human health or pose a significant threat to animal populations

PROVA ORALE N° 15

Estrazione degli acidi nucleici da un tessuto animale

Whole blood samples may be collected for haematology, clinical chemistry, toxicology, direct examination for bacteria or parasites, PCR testing, immunological testing, or for culture for bacteria or viruses.

PROVA ORALE N° 16

Cosa sono e a cosa servono le carte di controllo

Where investigating diseases of unknown cause multiple different specimens that represent the different stages of the disease progression in an animal or the population of animals (e.g. the pre-clinical, early clinical, active clinical, chronically affected and convalescent phases) should be collected

PROVA ORALE N° 17

Accreditamento di un laboratorio all'esecuzione di una prova

Surveillance of antimicrobial resistance and monitoring of the prevalence of, and trends in, resistance in bacteria from animals, food, environment and humans, constitutes a critical part of animal health and food safety strategies

PROVA ORALE N° 20

Elencare alcuni metodi di sterilizzazione in uso in un laboratorio di analisi

Receiving, unpacking and aliquoting specimens must be done in a way to avoid cross-contamination in order to guarantee reliable testing of samples and prevent exposure of personnel.

29 giugno - mattina

PROVA ORALE N° 1

Quali apparecchiature/strumenti sono necessari per eseguire un saggio enyme linked immunosorbent assays (ELISA).

Specimen reception areas should be equipped to facilitate the safe handling and processing of diagnostic submissions to avoid contamination of the work area, the personnel, cross-contamination among specimens and to allow easy disinfection in situations where specimen containers may have leaked.

PROVA ORALE N° 2

Cosa si intende per sensibilità e specificità analitica

Secretions can be collected directly into a vial or tube, or can be collected using swabs. Vesicular fluids provide a highly concentrated source of pathogen for diagnostic testing, and can be collected from unruptured vesicles using a sterile needle and syringe, and immediately transferred to a securely sealed vial or tube.

PROVA ORALE N° 3

Retro Trascrizione. Descrizione, uso.

The laboratory should be easy to clean, with surfaces that are impervious to water and resistant to chemicals used in the laboratory. There shall be a hand-wash basin, emergency shower, and eye wash station in each laboratory suite as appropriate for the chemicals and other hazards present

PROVA ORALE N° 4

Cosa si intende per multiplex PCR

Dependent on the suspected disease, condition of the carcass and facilities available for necropsies post-mortem specimens can be collected from one or multiple organs and submitted to the laboratory as either fresh (no preservative) or preserved specimens for further laboratory testing

PROVA ORALE N° 6

Elettroforesi – principi

Keep kit reagents on ice during reagent preparation steps.

It is recommended that all standards and samples be run at least in duplicate. Add 100 µl anti-“target” antibody to each well. Incubate for 1.5 hours at room temperature.

PROVA ORALE N° 8

Prevenzione dei rischi di cross-contaminazione nelle varie fasi di analisi di un campione con PCR.

Laboratory and animal facilities managers are responsible for providing a management system that ensures safe and secure handling, storage, and transport of these biological materials (a biological risk management system)

PROVA ORALE N° 9

Qualifiche del personale in un laboratorio accreditato

Real-time PCR and RT-PCR are highly sensitive techniques enabling amplification and quantification of a specific nucleic acid sequence with detection of the PCR product in real time. Quantification of DNA, cDNA, or RNA targets can be easily achieved by determining the cycle when the OCR product can first be detected

PROVA ORALE N° 10

Strumentazione in un laboratorio di diagnostica molecolare

Selection, collection, preparation, preservation and management of samples are critical variables in desing and development of an assay to ensure valid test results. Other variables such as transport, chain of custody, tracking of samples, and laboratory information management system are also key sources of variation/error that become especially important when the assay is implemented for routine testing.

PROVA ORALE N° 11

ELISA Enzyme linked Immunosorbent assay: principali tecniche

The fast procedure enables rapid processing of multiple samples and is easy to automate. The reduced number of handling steps results in high reproducibility from samples to sample and minimizes the risk of contamination since less manipulation is required

PROVA ORALE N° 12

Misure di contenimento in un laboratorio BSL3

The method for cell lysis needs to be carefully optimized so that the lysates provide similar performance in real-time RT-PCR as pure RNA templates. The method should preserve the gene expression profile and also prevent cellular and buffer components from interfering with amplification and detection.

PROVA ORALE N° 14

Metodi diretti e indiretti per il rilevamento virale

The samples will be provided as punch out from the FTA-CARD and can be kept at room temperature before reconstitution. The volume of the reconstituted samples (50 µl) will be sufficient to perform requested analysis. You can use the eluate directly in your PCR, there will be no need for RNA extraction.

PROVA ORALE N° 16

Differenza tra dispositivi di protezione individuale e collettivi

Most new real-time cyclers are installed with thermal-cycling modules that provide high ramping rates (i.e., fast heating and cooling capacities). This technology shortens the time to switch from one temperature to another, allowing faster run times in real-time PCR

PROVA ORALE N° 17

Importanza e tipologia dei controlli in un test diagnostico

We recommend that 1.25 units of GoTaq® DNA Polymerase be used per 50 µl amplification reaction. For most applications, enzyme will be in excess; the inclusion of more enzyme will not significantly increase product yield. Increased amounts of enzyme and excessively long extension times increase the likelihood of generating artefacts

PROVA ORALE N° 21

Il problema delle inibizioni in PCR, controlli e strategie operative

Remove the tube from the magnetic stand, add 300 µl of Wash Solution 1 to each sample, then vortex the sample in pulses for 20 to 30 seconds, being careful to prevent beads from sticking to the sides of the tube.

PROVA ORALE N° 24

Fasi pre-analitiche, gestione e verifica

The qPCR relies on analysis of fluorescent signal produced during amplification. The assays based on intercalating dyes are characterized by high sensitivity, as long as primers are Highly specific to the target sequence to avoid generation of non-specific products that would lead to overestimated or false positive results.

PROVA ORALE N° 25

Reazione di fissazione del complemento

The matrix in which the targeted analyte is found (serum, faeces, tissue, etc.) may contain endogenous or exogenous inhibitors that prevent some assays from working. This is of particular concern for enzyme-dependent tests such as polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA).