

# Glossary of Terms

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**Developed by**

International Seed Health Initiative (ISHI) of the International Seed Federation (ISF)

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## **GLOSSARY OF TERMS AND ITS PURPOSE**

The following glossary of terms document has been developed to facilitate the comprehension of the main terms used by the International Seed Health Initiative (ISHI).

## **DISCLAIMER**

The present glossary is a “living document” subject to periodic review and updates based on the work of the International Seed Health Initiative and in accordance with international organisations.

Term	Definition	Examples
Ad hoc working group	Group within ISHI that works on a specific topic that embraces many crops and has sufficient interest among ISHI TG. It is dissolved upon completion of the assigned tasks.	
Amplicon	A nucleic acid (DNA or RNA) sequence that serves as the source or product of amplification or replication by PCR. Synonym to “PCR Template”.	
Analytical sensitivity	Smallest amount of the target pathogen that can be detected with a given degree of confidence, commonly expressed as the limit of detection (LOD).	
Analytical specificity	The ability of an assay to detect the target pathogen(s) (inclusivity), while excluding non-target pathogens (exclusivity).	
Annealing temperature (Ta)	Temperature used in the annealing step in a PCR, which is highly dependent on the T <sub>m</sub> of the primers and probe(s) and used PCR reagents.	
Antimicrobial agent	A natural or synthetic substance that inhibits the growth of microorganisms or kills them.	
Artificially infected seed	Seeds that have been deliberately inoculated with a pathogen.	Seed inoculation through spiking or inoculation with ground material
Assay	A procedure for evaluating a seed sample for the presence or functional activity of a target pathogen (PCR assay, dilution plating assay, etc.). An assay consists of only one procedure, which includes sample preparation.	A seed health test is carried out using a method that is composed of one or more assays (e.g., SE-PCR, dilution plating or pathogenicity test), for which there is a detailed protocol.
Basic Local Alignment Search Tool (BLAST)	A sequence comparison algorithm optimized for speed used to search sequence databases for optimal local alignments to a query (Altschul <i>et al.</i> , 1990 and 1997; Fassler and Cooper, 2011).	
Best Practices	A guidance to encourage good laboratory practices. Not a synonym to “Guidelines” (see definition).	
Bioassay	An assay in which a plant tissue (e.g., seeds or leaves) that is suspected of being infected with a pathogen is applied to an indicator plant to assess viability/infectivity.	Infectivity and pathogenicity of Tobamoviruses are shown in the bioassay, and ELISA is used to confirm spread of the virus in the indicator plant.
Biochemical assay	An analytical procedure, typically used for bacteria, for evaluating the presence and functional activity of a target that is based on the different characteristics in their biochemical activities (e.g., primary, and secondary metabolisms).	Levan and esculin tests.

Term	Definition	Examples
Biological assay	Encompasses pathogenicity assay and bioassay (see definitions).	
Biological replicates	Samples drawn from the same original biological material prepared and tested simultaneously for pathogen presence.	
Bio-PCR method	A method that comprises of a dilution plating assay for the enrichment of the <b>pathogen</b> followed by a PCR assay with all DNA isolated from the organisms cultured on a given medium plate.	
Blotter assay	An assay, typically for the detection of fungi, that is based on the positioning of seeds on moistened blotter/filter paper, incubation under growth inducing conditions, and subsequent visual or microscopic examination/evaluation and/or molecular confirmation to assess pathogen growth.	
Chair	Member within ISHI TG that is responsible for leading the activities of a group, for presenting its work during the ISHI Technical Meetings, chairing conference calls, and for ensuring the progress of the group activities in a timely manner and to a high standard (with the help of the ISHI Technical Managers).	
Colony forming unit (CFU)	Unit used in microbiology that estimates the number of bacteria or fungal cells in a sample which are viable and able to multiply in controlled and conducive conditions. It is typically determined by dilution plating.	
Comparative Test (CT)	An evaluation of an assay or method that involves running replicate subsamples in multiple laboratories within pre-determined conditions and within a defined time frame to show an assay or method's reproducibility. Synonym to "Test performance studies" from EPPO terminology.	The comparative test showed that the method yielded reproducible results across all participating laboratories.
Complementary or Copy DNA (cDNA)	DNA synthesized from a single-stranded RNA template in a reaction catalysed by reverse transcriptase.	
Confirmatory test	An assay used for establishing the identity of a suspect pathogen after positive pre-screen assay results.	Dilution plating, pathogenicity test or grow out assays can be used for confirming results from SE-qPCR, ELISA, dilution plating or PCR assay.
Contamination	Presence in a commodity, in this case seeds, by contact or mixture of an undesirable material (i.e., contaminating pest, see definition) that makes the seeds unsuitable or unfit for use. Not interchangeable with infected or infested seed. Use only in the context of presence of impurities not considered as seed-borne pests.	
Contamination/infection level	Classification of a seed lot, ranging from healthy to high contamination/infection, which is based on the	<ul style="list-style-type: none"> <li>• High level: All subsamples should be detected positive with</li> </ul>

Term	Definition	Examples
	quantitative results of the assay and is related to the sensitivity and limit of detection (LOD) of the assay used to determine it. The level of contamination/infection does not imply that a seed lot is homogeneously or heterogeneously infected.	<p>relatively high values compared to the LOD.</p> <ul style="list-style-type: none"> <li>• Medium level: All subsamples should be detected positive with intermediate values compared to the LOD for homogeneous seed lots, or only a part of the subsamples tested are detected as positive with relatively intermediate/high values compared to the LOD for heterogeneous seed lots.</li> <li>• Low: All subsamples should be detected positive with values that are just above the LOD.</li> <li>• Healthy: All subsamples should be detected negative (below the LOD)</li> </ul>
Contamination/infection rate	Number of seeds detected as contaminated/infected out of the total number of seed tested and expressed in percentage, which is based on the quantitative results of the assay	
Contaminating pest	Category of organism that can be present in a seed lot but that is not seed-borne and includes inert matter and seed of other plants (e.g., weed, parasitic plant) as pest.	Mixing of crop seeds with weeds.
Conventional PCR	Type of PCR assay where the amplicon is visualized after completion of the PCR, generally by (gel-) electrophoresis. Synonym to “End-point PCR”.	
Country lead	Member within ISHI TG nominated by the ISHI TG in the country when there are three or more ISHI members from the given country. Responsible for coordinating the activities of the TG of the country, keeping them informed of developments in ISHI and for bringing country specific issues to the attention of ISHI at the appropriate meetings and conference calls. Ensure the active participation of every company/laboratory in method development and validation.	
Cycle quantification (Cq)	The number of cycles in real-time PCR that were needed for the fluorescence to reach a quantification threshold. Note: “Cq” is the preferred term in the MIQE guidelines (Bustin <i>et al.</i> , 2009) and preferred over “Cycle threshold” (Ct).	
Cycle threshold (Ct)	Synonym to “Cq”, which is the preferred term.	
Cut-off	Level that is used as decision threshold for a positive result based on experimental data.	Number of cycles of a qPCR or absorbance in an ELISA.
Degenerate primer	Primer that targets a collection of unique sequences. Some of the positions of a degenerate primer comprise	

Term	Definition	Examples
	of has several possible bases. It can be used when no conserved sequences are present in the target, or in the case when several targets are investigated.	
Detection	Determining the presence of the target pathogen or molecules associated with this pathogen, through evidence of one of the following: symptoms, morphology, or specific molecules (e.g., nucleic acid, proteins).	Xcc DNA was detected using the SE-PCR assay.
Diagnostic performance	An evaluation of the ability of the assay to discriminate between positive and negative seed lot samples.	
Diagnostic sensitivity	Measure of the confidence of the assay that a positive result is a true positive.	
Diagnostic specificity	Measure of the confidence of the assay that a negative result is a true negative.	
Diagnostic testing	The detection or identification of a pathogen from both symptomatic and asymptomatic samples.	
Diagnosis	The identification of the nature of a disease or other problems based on the symptoms, the seed history, and the analytical results.	
Dilution plating assay	An assay in which a seed extract is plated onto semi-selective media that allow for the cultivation of the target pathogen and its evaluation by visual examination/evaluation to assess the pathogen's growth and morphology.	
Direct test	An assay or a combination of assays, which demonstrate the presence, viability, and pathogenicity of a pathogen on and/or in the seed.	The combination of the dilution plating and pathogenicity assays in the Cmm method constitute direct tests in which bacteria are isolated from seeds and evaluated for their ability to cause disease.
End-point PCR	PCR assay where the amplicon is visualized after completion of the PCR, generally by (gel-) electrophoresis. Synonym to "Conventional PCR".	
Enzyme-linked immunosorbent assay (ELISA)	An assay that detects antigens from a specific organism by the process of immobilisation on a surface using antibodies directed against the protein to be measured.	
Exclusivity	Ability of a method or assay to discriminate (no detection) non-target pathogens and contaminants.	
GC content	The rate of nitrogenous bases in a DNA or RNA molecule that are either guanine (G) or cytosine (C).	
Gradient PCR	Type of PCR that tests multiple annealing temperatures within the same PCR run to determine the optimal annealing temperature in a PCR assay.	
Grow-out assay	An assay in which seeds from a seed sample are sown under disease-conducive conditions, and plants are subsequently examined for the presence of disease	

Term	Definition	Examples
	symptoms caused by the pathogen to demonstrate seed to seedling transmission of a given pathogen.	
Healthy seed	Seed lot for which the sample was found to be negative for all target pathogens tested via a seed health test.	
Heterogenous	Seed lot for which tested subsamples are giving variable test results when tested multiple times with the same assay.	
Homogeneity test	Test that aims at demonstrating the homogenous distribution of pathogen in samples. This test is performed on multiple subsamples from a single seed lot.	
Homogenous	Seed lot for which all tested subsamples are giving comparable test results when tested multiple times with the same assay.	
Identification	Determining the identity of a detected suspect pathogen.	Colonies isolated from Brassica seed can be identified as Xcc or Xcr using PCR, or by examining symptoms in a pathogenicity assay.
Identification PCR	A PCR assay whereby the nucleic acids used as template originate from single isolate microbial cultures to determine if this isolate is of the target pathogen.	
<i>In silico</i> analysis	Experiment performed on a computer or via computer simulation software.	
Inclusivity	Ability of a method or assay to detect the target pathogen regardless of its genetic diversity, geographical origin, or diversity of hosts.	
Incubation	Conditions for culturing the pathogen of interest, including media type, atmosphere, temperature, and duration.	Bacteria are typically cultured on semi-selective media, in the dark, at 28 °C for several days.
Indirect test	Assay which demonstrates the presence of molecules (proteins or nucleic acids) indicative of the pathogen but does not demonstrate its viability or pathogenicity.	The CGMMV ELISA assay is an indirect test, which evaluates for the presence of proteins associated with the virus on a seed sample.
Infection	Presence in a commodity, in this case seeds, of a living pathogen that enters, invades, or penetrates and establishes a parasitic relationship with a host plant (APS, 2022).	
Infestation	Presence in a commodity, in this case seeds, of a living pest of the plant or plant product concerned. Infestation includes infection (IPPC-FAO, 2022).	Includes insects and pathogens.
Inoculation	Act of introducing a suspect pathogen to a medium or a susceptible plant to assess the viability and/or pathogenicity of this pathogen.	
ISHI Technical Group (TG)	All plant pathologists or other appropriate staff designated by ISHI Members to participate in the activities of ISHI.	

Term	Definition	Examples
Isolate	A single organism, such as a bacterium, fungus, or virus, that has been separated from a mixed population and is being cultured independently.	
ITG Chairs and Country Leads Group (CCL)	Group within ISHI that critically reviews project proposals, including the key factors for the ISHI projects, and verifies that proposals take into consideration best practices identified by ISHI and adhere to positions taken by the industry on Seed Health. Consulted on topics that concern ISHI as a whole.	
ISHI Members	Seed companies, public sector institutions and service laboratories that run a seed health testing laboratory. Together, they develop internationally recognized reference methods for seed health testing. Membership includes a fee dependent on the companies' turnover.	
Limit of detection (LOD)	Smallest amount of the target pathogen that can be detected with a given degree of confidence. Evaluated with "Analytical sensitivity" experiments.	
Locked nucleic acid (LNA) Probe	A modified RNA monomer, which contains a methylene bridge bond linking 2' oxygen to the 4' carbon of the RNA pentose ring. Thermal stability of annealed oligonucleotides and specificity of the oligonucleotide to the target sequence are increased. In case of qPCR, signal/noise ratio and T <sub>m</sub> are increased compared to a conventional PCR.	
Look-alike	A non-target organism that resembles the target organism based on its morphology on (semi-selective) growth media or molecular features.	
Matrix	All compounds in a given sample which are not the analyte detected in the assay (e.g., seeds or tissues).	Due to varying amounts of PCR inhibitors on different seed lots, a matrix effect on the efficiency of the qPCR can be observed.
Melting temperature (T <sub>m</sub> )	Temperature in a PCR at which half of the DNA duplex will dissociate to become single-stranded.	
Method	A collection of one or more assays that together show the presence, viability, and pathogenicity of a pathogen. A method is a description of how a seed health test is conducted.	A seed health test is carried out using a method that is composed of one or more assays (e.g., SE-PCR, dilution plating or pathogenicity test), for which there is a detailed protocol.
Method description	A detailed step by step description of the assays of a given method. Preferred term: "Protocol".	
Method validation/Validation	Process that determines the fitness of a method for its intended purpose. Assessment of the method within ISHI is based on six performance criteria: analytical specificity, analytical sensitivity, selectivity, repeatability, reproducibility, and diagnostic performance.	

Term	Definition	Examples
Method Validation Team (MVT)	Group within ISHI that ensures ISHI seed health methods are compliant with the latest version of ISHI technical guidelines for the validation of seed health tests and are ready for publication. The MVT is also responsible for periodically evaluating these guidelines for their use within ISHI, monitoring those of other accreditation bodies and updating them when necessary.	
Microscopic examination/evaluation	Examination/evaluation of pathogens by means of a microscope to provide an enlarged image of the pathogens, which are not visible or identifiable with the naked eye.	
Minor groove binder (MGB) probe	Dual-labelled 5' hydrolysis probe consisting of a 5' fluorescent reporter dye and a 3' quencher conjugated to a MGB moiety. The hydrophobic binding of the MGB to the DNA helix increases the T <sub>m</sub> by 15 °C–30 °C, which allows using shorter probes with high specificity.	
Mismatch	Non-complementary base pairing on DNA strands of pairs other than A-T and G-C.	
Multiplex PCR	A single PCR that consists of multiple primer and probe sets amplifying several distinct targets (In contrast to singleplex, monoplex).	
Naturally infected seed	Seed that has become infected with a pathogen through natural processes, resulting in diseased seedlings when planted under disease conducive conditions.	
Negative sample	Sample in which the absence of a given target pathogen has been shown via a seed health test.	
Negative seed (lot)	Seed lot for which the sample was found to be free from a given target pathogen via a seed health test.	
Non-target	Organisms that the method is not intended to detect.	
Pathogen	A bacterium, virus, fungus, or other microorganism that can cause a diseased state to a given plant.	
Pathogenicity assay	An assay in which a pure culture of a putative pathogen isolated from a seed lot is inoculated onto a suitable host plant under disease-conducive conditions to assess the development of typical disease symptoms.	In the Xcc pathogenicity assay, cabbage seedlings of a susceptible cultivar are inoculated with the putative Xcc culture to determine if the isolate is pathogenic.
PCR template	A nucleic acid (DNA or RNA) sequence that serves as the source or product of amplification or replication by PCR. Synonym to "Amplicon".	
Peer-review	Evaluation of documents or reports by ISHI TG other than the producers of the work.	
Performance criteria	Set of analytical and diagnostic metrics required for the validation of an assay or method to ensure the assay/method is fit for purpose.	
Performance specifications	Requirements for a performance criterion according to which it can be judged that the assay or method is fit for the intended purpose.	

Term	Definition	Examples
Pest	Any species, strain or biotype of plant, animal, or pathogenic agent that can cause injuries to plants or plant products (IPPC-FAO, 2022).	
Plating assay	An assay, typically for the detection of fungi, that is based on the positioning of seeds on (non-selective or semi-selective) agar plates, incubation in fungal growth inducing conditions, and subsequent visual or microscopic and/or molecular evaluation(s) to assess pathogen growth.	
Polymerase Chain Reaction (PCR) assay	An assay that assesses the presence of nucleic acid from specific organism(s) by the process of multiplication (amplification) of small nucleic acid templates (PCR product) relative to positive and negative controls.	
(Genetic) polymorphism	The presence of two or more variants of a specific DNA/RNA sequence among different individuals or groups within a population.	
Position paper	A paper which formalizes ISF/ISHI opinion on an international issue critical to ISF/ISHI members and meant to be used publicly.	
Positive sample	Sample in which the presence, viability and pathogenicity of the target pathogen have been confirmed via a seed health testing method. Seed that has been tested with a direct test and resulted in the development of disease symptoms is considered positive. If no direct test is available, a second indirect test based on a different principle should be used.	
Positive seed (lot)	Seed lot in which the presence, viability and pathogenicity of a given target pathogen has been shown via a method.	
Pre-Comparative Test (CT)	Test performed prior to a CT for CT participants to get accustomed to an assay/method.	
Pre-screen assay	An indirect assay that is used to identify negative and suspect seed samples. A negative result is conclusive and exempts seed lots from further testing. A positive pre-screen result is indicative of a sample suspected to be infected, and further testing with a direct test to demonstrate pathogen viability and pathogenicity is required to conclusively determine the health status of the seed lot.	The Acidovorax SE-PCR pre-screen assay is used to rapidly evaluate seed lots for the presence of target DNA and identify which seed lots require further testing using a direct test.
Primer	Short, single-stranded, and specific DNA oligonucleotide that is used in PCR by hybridization with DNA or RNA from a sample in a PCR assay.	
Primer dimer	Unintended PCR product amplified from annealing of primers with the same sequence (self-dimer or homodimer) or with different sequences (heterodimer).	
Probe	Short, single-stranded DNA oligonucleotide that carries a fluorescent label and is used to detect the presence of	

Term	Definition	Examples
	a specific DNA fragment in a PCR mixture through the hydrolysis of the probe during elongation and separation from the quencher in a qPCR assay.	
Proficiency test (PT)	An evaluation of a laboratory's ability to perform its own protocol by testing blind samples and comparing the results to the pre-defined specifications.	
Project lead	Participant within ISHI TG responsible for leading a specific ISHI project and for informing the ITG of the progress made.	
Protocol	A detailed step by step description of the assays of a given method.	A seed health test is carried out using a method that is composed of one or more assays (e.g., SE-PCR, dilution plating or pathogenicity test), for which there is a detailed protocol.
Quantitative real-time PCR assay (qPCR)	A PCR assay for which amplification of the genetic material is measured by for example a fluorescent dye in real time, enabling quantitation of the PCR product.	
Quarantine pest	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and that is being officially controlled (IPPC-FAO, 2022).	
Quencher	A molecule that absorbs and suppresses a fluorescent signal.	
Regulated non-quarantine pest	A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (IPPC-FAO, 2022).	
Regulated pest	A quarantine pest or a regulated non-quarantine pest (IPPC-FAO, 2022).	
Repeatability	Degree of similarity in results of replicates of the same seed subsamples when the assay is performed with minimal variations in a single laboratory.	
Reproducibility	Degree of similarity in results when the assay is performed across laboratories. Reproducibility is evaluated in a "Comparative Test".	
Reverse transcription (RT)	The process of converting RNA to cDNA, using a reverse transcription enzyme, targeted primers (or random hexamers) and dNTPs.	
Reverse transcription-qPCR (RT-qPCR) assay	A qPCR assay for which the template material is RNA.	
Saprophyte	Organism found on the seed and does not cause any disease to the plant.	
Seed (as a commodity)	Seeds (in the botanical sense) for planting (IPPC-FAO, 2022).	

Term	Definition	Examples
Seed-borne pest	A pest carried by seeds externally or internally that may or may not be transmitted to plants growing from these seeds and cause their infestation (IPPC-FAO, 2017) (includes infection).	
Seed disease	Disease that is caused by a bacterium, virus, fungus, or other micro-organisms, which may be on and/or in the seed at planting or present in the soil.	
Seed extract (SE)-qPCR	An assay in which a seed extract is used for isolation of the nucleic acid of the pathogen present on or/and in the seed and the subsequent evaluation of the nucleic acid by qPCR assay.	
Seed health test	An evaluation for the presence of a pathogen in a seed sample via a method.	A seed health test is used to evaluate a tomato seed sample for the presence of Cmm.
Seed sample	A portion of a seed lot taken for testing purposes. A sample must be taken in a manner to ensure that it is representative of the entire seed lot from which it was taken (e.g., following ISTA rules on seed sampling).	
Seed surface sterilization	Step of an assay (typically with e.g., ethanol, sodium hypochlorite) that may be used to minimize the growth of saprophytic microorganisms.	
Seed-transmitted pest	A seed-borne pest that is transmitted via seeds directly to plants growing from these seeds and causes their infestation (IPPC-FAO, 2017).	
Seed treatment	Physical or chemical process that aims for the disinfection/disinfection of a seed lot.	Chemical processes include acid extraction, calcium or sodium hypochlorite, tri-sodium phosphate.
Selectivity	The effect of different seed matrices on the ability of the assay to detect the target pathogen(s).	
Sequence alignment	Comparison of two or more protein, DNA, or RNA sequences to evaluate sequence similarity.	
Serological assay	An indirect assay that is used to determine the presence of a given pathogen using antibodies for the recognition of specific antigens from this pathogen.	ELISA is a type of serological assay.
Single nucleotide polymorphisms (SNPs)	DNA sequence variants at a single base locus.	
Stability test	Test that aims to demonstrate that the infection of a given seed lot does not undergo any significant change over the course of the comparative test (CT). This test, typically performed by the CT organiser, after the deadline for participating to the CT, is carried out on a complete set of samples used for the CT.	
Strain	A genetic mutation of a microorganism, such as a bacterium, fungus or virus. Strains may exhibit differences in characteristics, such as virulence, antibiotic resistance, or metabolic capabilities.	

Term	Definition	Examples
Subsample	A portion of the seed sample. Typically, a sample is divided into multiple subsamples for testing, in correlation with the detection limit of the method used.	For the detection of viruses in Solanaceae, the sample size is 3,000 seeds and the subsample size is 250 seeds, since the detection limit of ELISA assay is one infected seed in 250 non-infected seeds.
Suspect sample	A sample which yielded a positive result in an indirect assay, for which viability and/or pathogenicity of the detected target have not been demonstrated.	
Sweat box grow-out assay	Type of grow-out assay in which seeds from a seed sample are sown, typically in vermiculite, under disease-conducive conditions in a closed plastic box that leads to high humidity. Plants are subsequently examined for the presence of disease symptoms caused by the pathogen.	
Target	Pathogens that the method is intended to detect.	
Technical fact sheet	A list of questions and answers and/or factual data relating to a particular subject especially those giving basic information on ISHI positions in relation to specific aspects on Seed Health testing and methods.	
Technical group (TG)	Employees of the International Seed Health Initiative (ISHI) members participating to the initiative.	
Technical guidelines	A guidance to encourage good seed health testing practices. Not a synonym to “Best Practices” (see definition).	
Technical opinion paper	Paper developed by ISHI that reflects the opinion of the group on a specific topic.	
Technical replicates	Number of repetitions prepared from one sample for an assay (e.g., duplicate, or triplicate reactions during ELISA or PCR tests).	
Threshold	The limit at which measurements are determined to indicate positive/suspect or negative results (e.g., during ELISA or qPCR).	The threshold used for ELISAs is typically set at 2.5 times the average value of the negative control.
Validation (of a method)	Process that determines the fitness of a method for its intended purpose. Assessment of the method within ISHI is based on six performance criteria: analytical specificity, analytical sensitivity, selectivity, repeatability, reproducibility, and diagnostic performance.	
Validation plan	Document that outlines how and which elements will be assessed to determine that a method is fit for its intended purpose. It also includes the performance specifications.	
Validation report	Document that contains the compilation of the results and their analyses obtained during the evaluation of a	

Term	Definition	Examples
	method to determine that it is fit for its intended purpose. It is a follow-up from the validation plan.	
Variant	A strain with an alteration in the DNA sequence.	
Vice-chair	Participant within ISHI TG that is responsible for reviewing the agenda with the Chair and helps the Chair with the minutes review and substitute the Chair when needed.	
Visual examination/evaluation	The examination/evaluation of pathogens that are visible and can be identified by means of a naked eye.	

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