

Innovative design, smart combination...
all the advantages of Molecular Biology in a quick detection

Speed-oligo[®]
Quick Visual Oligochromatography



Speed-oligo[®] allows for a visual and direct analysis, through a simple technique which provides results in just a few minutes. This PCR-based method coupled to a dipstick device enables a **quick, highly sensitive and specific detection**.

www.vircell.com



Available products

Multitests

Bacterial meningitis

Cat. No.		Pack size
SP006	SPEED-OLIGO [®] BACTERIAL MENINGITIS 3 test lines for <i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> and <i>Neisseria meningitidis</i>	24 tests

Bordetella pertussis

Cat. No.		Pack size
SP011	SPEED-OLIGO [®] BORDETELLA 2 test lines for <i>B. pertussis</i> / <i>B. holmesii</i> and <i>B. pertussis</i>	40 tests

Influenza A

Cat. No.		Pack size
SP015	SPEED-OLIGO [®] NOVEL INFLUENZA A H1N1 2 test lines for influenza A and pandemic influenza A H1N1	40 tests

Mycobacteria

Cat. No.		Pack size
SP005	SPEED-OLIGO [®] MYCOBACTERIA* 7 test lines for <i>M. tuberculosis</i> complex, <i>M. avium</i> /intracellular/scrofulaceum complex, <i>M. chelonae</i> /abscessus complex, <i>M. fortuitum</i> , <i>M. kansasii</i> , <i>M. goodii</i> and <i>Mycobacterium</i> genus (For culture identification)	40 tests
SP016	SPEED-OLIGO [®] DIRECT MYCOBACTERIUM TUBERCULOSIS* 2 test lines for <i>Mycobacterium</i> genus and <i>M. tuberculosis</i> complex (For direct samples)	40 tests

Single tests

Chlamydomphila pneumoniae

Cat. No.		Pack size
SP003	SPEED-OLIGO [®] CHLAMYDOMPHILA PNEUMONIAE	40 tests

Group B Streptococcus

Cat. No.		Pack size
SP004	SPEED-OLIGO [®] GROUP B STREPTOCOCCUS*	40 tests

Legionella pneumophila

Cat. No.		Pack size
SP002	SPEED-OLIGO [®] LEGIONELLA PNEUMOPHILA	40 tests

Mycoplasma pneumoniae

Cat. No.		Pack size
SP001	SPEED-OLIGO [®] MYCOPLASMA PNEUMONIAE	40 tests

Combi products

Bacterial pneumonia

Cat. No.		Pack size
SP008	SPEED-OLIGO [®] BACTERIAL PNEUMONIA COMBI 3 types of strips for <i>Chlamydomphila pneumoniae</i> , <i>Legionella pneumophila</i> and <i>Mycoplasma pneumoniae</i> (3x8 tests)	24 tests

* reagents for a rapid extraction included in the kit

Innovative design, smart combination...

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Speed-oligo[®]

Quick Visual Oligochromatography

vircell 
MICROBIOLOGISTS

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Latest tendencies for the diagnosis of infectious diseases

Nowadays the tendency for the diagnosis lies in the use of more recent and sensitive techniques for antigen detection and nucleic acids research, in order to obtain a quicker diagnosis. This tendency aims to develop more effective treatments and thus turns fast, sensitive and specific identification into a need.

Some time ago, pathogens isolation was the standard technique used as a reference for the rest of diagnosis trials. However, it is no longer the most sensitive method because of the development of the new Molecular Biology techniques. Owing to the fact that Molecular Biology allows amplifying only a certain infectious agent, sensitivity is increased without decreasing specificity. Furthermore, Molecular Biology provides some other advantages: the process is usually faster and, as a consequence, diagnosis can be available in time to influence patient care. Besides, it is a less laborious and more economical process. The use of Molecular Biology techniques also avoids the use of specific culture systems, for example, there is no need of using several cell lines for the optimal detection of a virus.

Within Molecular Biology, PCR stands out as the most powerful tool for diagnosis. However, up to date, the main disadvantage of the detection through nucleic acids and especially through PCR lies in the revealing, -due to the necessity of specialised staff to interpret the results-, the required time, the necessity to handle toxic products and the excessive manipulation.

Applications of Molecular Biology to diagnosis

alternatives to the detection of PCR

More and more biotechnology companies have started to design new diagnostic tools based on Molecular Biology techniques. Nowadays, different technologies applied to the detection of nucleic acids can be found in the market:

Nucleic acid electrophoresis: Possibly the most traditional and widely used method for the detection of nucleic acids.

PCR-ELISA: It detects PCR products through an automated technique such as ELISA. This technology is based on the hybridization of PCR products with marked probes.

PCR-Line blot: In this technique detection of PCR products is visualized on a strip. Its peculiarity is that it can be coupled to the detection of multiple PCR. Excessive manipulation and time (2 hours) are required for the assay.

Real time PCR (qPCR): It is probably the most advanced technology because the result is visualized directly in the computer. However, this technique is quite expensive to be introduced.

Speed-oligo® (quick visual oligochromatography): PCR revealing technique through a quick detection. This technology, recently developed by Vircell, allows a visual and direct analysis so that results can be obtained within a few minutes. Furthermore, interpretation of the result is very easy, even easier than real time PCR.

Alternatives to PCR revealing

	Electrophoresis	Southern blot	ELISA	Line blot	qPCR probes	Speed-oligo®
Specificity	↓	↑	↑	↑	↑	↑↑
Cost	↓	↑↑	↑	↑	↑↑	↓
Time	1h	overnight	2-4h	2-4h	0	10 min
Equipment: cost	↓	↑↑	↑	↓	↑↑	↓↓
Equipment: exclusive	Yes	Yes	No	No	Yes	No
Technical complexity	☹	☹	☹	☺	☹	☺☺
Manipulation	☹	☹	☹	☹	☺☺	☺
Hazards	⚠	⚠	↓	↓	↓↓	↓↓
Suitable for automation	☹	☹	☺	☹	☺☺	☹
Suitable for multitest	☹	☹	☹	☹	☹	☺

Speed-oligo® is the most specific technique due to its double hybridization

The oligo probes -for specific and control amplicon- are immobilized in the membrane. The detection method is done by means of a double hybridization: on one hand with the immobilized probes in the membrane and on the other hand with a colloidal gold marker. The double hybridization avoids the detection of unspecific amplified fragments which would lead to false positives in any other technique.

Speed-oligo® is one of the most affordable techniques of Molecular Biology

Since its creation, Speed-oligo® was conceived as a technique that brings the advantages of Molecular Biology closer to the daily work in most of the laboratories. Taking into account the distribution channel for this kind of products, Vircell recommends its distribution network to establish an end-user price affordable for all laboratories.

Speed-oligo®- minimal equipment

This new development is also considered an affordable technique because of the little equipment required. It is possible to assay the complete technique just by using a thermocycler and thermoblock, very usual instruments in current laboratories. Moreover, these instruments can also be used for other techniques conducted in the lab, avoiding room space problems.

Speed-oligo® stands out because of its speed

In just 5 minutes hybridization of the strips takes place and it is followed by an easy reading and interpretation thanks to the reading and interpretation card included in the kit.

Speed-oligo® is a very easy technology that requires little manipulation

Vircell R+D Department has aimed at the highest performance features maintaining the easy performance of the technique. Neither specific knowledge nor previous experience to conduct or interpret the technique are required.

Maintaining the lowest level of manipulation was another important goal of the team that has developed Speed-oligo®. The product contains all the necessary reagents to conduct the technique including prepared PCR mix. This characteristic implies an important reduction in time and avoids possible contamination.

Speed-oligo® avoids using hazardous substances

All the advantages of Molecular Biology are included in a quick detection, avoiding the use of ethidium bromide. This new technology solves the revealing and interpretation of the PCR technique in an easy and fast way.

Speed-oligo® offers possibilities for the multitest diagnosis

This technology is characterized by double hybridization, which allows the diagnosis of different parameters in the same strip. This concept may result very useful in some cases such as the identification of mycobacteria or the detection of the bacterial meningitis causing agents.

Searching for market opportunities

a technology designed for a specific type of client

"...the implementation of the PCR in microbiology laboratory care has been restricted to a small group of infectious agents of great economic interest to the companies of microbiological diagnostics, such as virus of the human immunodeficiency (HIV), hepatitis C (HCV), hepatitis B virus (HBV) or cytomegalovirus, for which have been marketed systems well standardized and, to a greater or lesser extent, automated.

For other infectious agents with lower economic interest, with no commercial kits to carry them out or, if any, are not easy to apply, methods not marketed are used, optimized in the laboratories. However, in general, its use has been limited to reference centres or large hospitals."

Costa, J. 2004. Reacción en cadena de la polimerasa (PCR) a tiempo real.
Enferm Infecc Microbiol Clin 22 (5): 299-305

Each laboratory has its own methods and these may sometimes break the minimum repetitivity and reproducibility conditions, which are necessary for the diagnosis in assistential centres. Speed-oligo[®] covers this need by providing a standardized method that includes, in just one CE marked product, all the necessary reagents to guarantee the specific and fully sensitive diagnosis common in Molecular Biology techniques.

Speed-oligo[®] covers several parameters of interest for the assistential diagnosis that are not provided by other companies or, if provided, do not offer the simplicity, speed and consistency features required by the client.

Potential users

customers interested in Speed-oligo[®] technique

- Diagnostic labs that want to incorporate Molecular Biology techniques to their routine work. We offer a simple technique for those clients that are not familiarized with the molecular diagnosis.
- Labs using PCR techniques that need a fast and simple detection method with the maximum reproducibility, sensitivity and specificity.
- Labs in emergency services.
- Labs in reference centres that are looking for a commercial alternative to home-made PCR or that are processing exotic parameters that are not worth a RT-PCR due to the cost and expiry date of the kits.

Status of PCR in clinical laboratories

limited use in diagnostic laboratories

Despite its high diagnostic potential and its invaluable technical qualities, PCR has often a limited use in the assistential routine of the clinical microbiology laboratories. These are some of the reasons for this situation:

- Lack of linearity with the initial concentration of target: real time PCR.
- Global extraction time + PCR + detection: 2 working days.
- Difficulty in the extraction techniques.
- Difficulty in the detection techniques.

Some advances, as commercial kits for extraction or qPCR, have palliated some of these problems. Others, as the duration or difficulty in the detection processes, are still present.

Being aware of this reality, Speed-oligo[®] is born to bring the Molecular Biology techniques closer to the assistential diagnosis. Providing reproducibility, sensitivity and specificity, Speed-oligo[®] includes all the necessary reagents for PCR reaction and strip detection. In just 5 minutes it shows visual results that can be easily interpreted, even easier than real time PCR.

Technical fundamentals of Speed-oligo®

a simple technique full of possibilities

Based on a PCR technique, Speed-oligo® provides all the advantages of Molecular Biology in a quick detection, allowing immediate and highly sensitive and specific results.

This new technique, developed by Vircell, takes place in 3 steps: DNA extraction, amplification of the specific oligo pair (PCR) and detection of the amplified product with the strip.

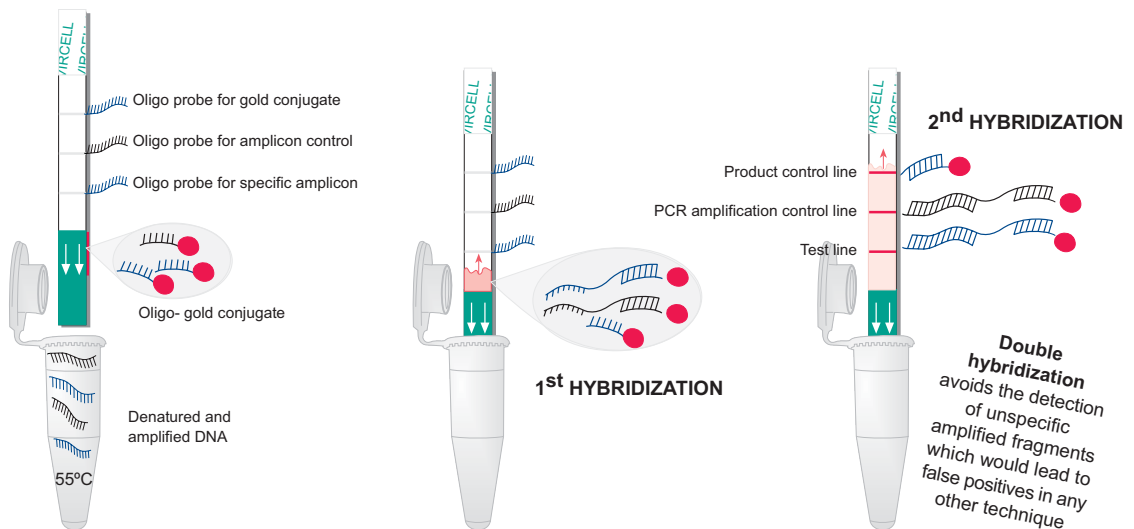
Speed-oligo® contains all the reagents necessary to perform the PCR reaction and the strip detection.



The denatured PCR products (specific microorganism amplicon and control amplicon) are diluted in the hybridization solution and come into contact with the strip.

When flowing along the strip, the specific and control amplicons react with the complementary probes coupled to colloidal gold.

When reaching the visualization zone, the complexes of specific and control amplicons and the colloidal gold conjugates, react with a second probe which is bound to the membrane, leading to a coloured band. The excess of conjugate reacts with a complementary probe on the control line.



Parameters of interest

available products in Speed-oligo® technique

- ***Mycobacterium tuberculosis*** (Mtb) is the etiological agent of tuberculosis (TB) in humans. TB killed more than 2,000,000 people worldwide in 2006, more than AIDS, malaria, and other tropical diseases combined did.

One third of the world's population is infected with tuberculosis, which is responsible for 26% of preventable deaths. The genus *Mycobacterium* includes a broad group of environmental microorganisms found in habitats such as water or soil. However, a few are pathogens of animals and humans. Mtb complex includes *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*, all casual agents of tuberculosis. *Mycobacteria* other than tuberculosis (MOTT) include a group of opportunistic pathogens that can produce lung-resembling TB, lymphatic, tissue and disseminated disease, but are acquired from the environment as person to person transmission rarely occurs. Distinguishing infections by each group has important epidemiological and public health implications. The reference techniques for the investigation of *M. tuberculosis* are microscopy and culture. Microscopy is relatively simple but lacks sensitivity and several samples are required. There has to be a high bacterial concentration (5000-10000 bacilli per ml of sputum) in order to be detected. Due to its low sensitivity, microscopy cannot be used to rule out infection until culture results are available. In addition, acid-fast direct smear microscopy does not enable to differentiate between Mtb complex and MOTT infections. Culture has an excellent sensitivity, but the slow growing rate of mycobacteria implies a delay in results to up to eight weeks. The use of PCR in TB detection can shorten this time to one day with an excellent sensitivity.

SPEED-OLIGO® MYCOBACTERIA (ref.SP005)- culture identification

SPEED-OLIGO® DIRECT MYCOBACTERIUM TUBERCULOSIS (ref. SP016)- direct detection

- ***Mycoplasma pneumoniae*** is a common pathogen causing around 10-30% of the community-acquired pneumoniae cases. Most of them are relatively mild but they can also induce acute, even fatal symptoms. Pneumonia caused by *M. pneumoniae* is more frequent in infants and teenagers. Culture isolation of pathogens and serological test detection have been the classic diagnostic methods but the fastidious nature of this pathogen as well as the high seroprevalence are the major disadvantages of these procedures. PCR is used as the detection method of *M. pneumoniae* in respiratory samples replacing other direct diagnostic methods due to its high sensitivity based on amplification. This enables pathogen detection in respiratory secretions during the early disease phase, when antibody response has not appeared.

SPEED-OLIGO® MYCOPLASMA PNEUMONIAE (ref. SP001)

SPEED-OLIGO® BACTERIAL PNEUMONIA (ref. SP008)

- ***Chlamydia pneumoniae*** is an obligate intracellular bacteria related to a wide variety of acute and chronic diseases such as pneumonia and bronchitis. Approximately 10% of the community-acquired pneumonia cases are associated with this pathogen. The respiratory infections with *C. pneumoniae* occur worldwide and affect all age groups. Seroepidemiological studies show that between 50 and 75% of adults have antibodies against *C. pneumoniae*. Most of the people suffer infections and reinfections through their lives.

SPEED-OLIGO® CHLAMYDOPHILA PNEUMONIAE (ref. SP003)

SPEED-OLIGO® BACTERIAL PNEUMONIA (ref. SP008)

- Even though more than 50 species have been described, ***L. pneumophila*** serogroup 1 induces more than 90% of the cases of Legionnaires' disease. Direct diagnosis methods include culture, direct fluorescent staining and antigen detection in urine.

While the two first methods present a variable and low sensitivity, the last one has turned into the reference technique in most of the laboratories. However, antigenuria is not always present in all patients and using just this diagnostic assay could lead to not diagnosing up to 40% of the legionellosis cases.

The detection of *L. pneumophila* genetic material in sputum, urine or blood based on the PCR techniques has been successfully used in investigation and reference laboratories. Since most of the rapid tests only detect infections caused by *L. pneumophila* serogroup 1, one PCR test that includes all the serogroups could increase the capacity to diagnose these infections.

SPEED-OLIGO® LEGIONELLA PNEUMOPHILA (ref. SP002)

SPEED-OLIGO® BACTERIAL PNEUMONIA (ref. SP008)

- Last April 2009, WHO announced the identification of a **new virus of influenza A**. This nH1N1 strain has not circulated in human beings before. It is a completely new virus with no previous immune response. The virus is contagious and spreads easily among people and also among countries. Worldwide more than 214 countries and overseas territories or communities have reported laboratory confirmed cases of pandemic influenza H1N1. Until now, only sequencing and RT-PCR targeting the specific sequence of this new virus are able to differentiate the novel virus from the current circulating seasonal strains. Only a limited number of centres have these tools. Therefore, a new sensitive and specific diagnostic method, suitable for any laboratory, is necessary.

SPEED-OLIGO® NOVEL INFLUENZA A H1N1 (ref. SP015)

- *Bordetella pertussis* is the causal agent of whooping cough, a contagious disease propagated worldwide that mainly affects infants between 0 and 4 years old and is particularly severe in newborns. Currently, pertussis represents a major public health problem due to the loss of efficiency of the vaccine and the lower vaccine coverage in some communities, atypical presentations that escape the clinical suspicion by doctors and the changing age range of the affected population. The reference technique for the investigation of *Bordetella* used to be isolation through culture, despite it was a complex and low sensitive technique. Serology, though reliable, is based on seroconversion and requires an acute and a convalescent serum, which is not always available; besides, it is not suitable to distinguish between infection and vaccination. As a consequence, PCR is becoming the reference technique for laboratory pertussis diagnosis and is currently accepted as a proof of infection in many countries with mandatory notification systems.

SPEED-OLIGO® BORDETELLA (ref. SP011)

- **Group B streptococcus** (GBS) infection remains one of the most frequent causes of neonatal morbidity and mortality, even though its incidence has decreased due to the routine antenatal screening. For the detection strategies based on the routine screening of pregnant women, it is extremely important to have techniques with maximum level of detection. A quick method for detecting GBS could help identifying colonized children with risk of invasive disease so that they can be properly monitored.

SPEED-OLIGO® GROUP B STREPTOCOCCUS (ref. SP004)

- Despite the fact that efficient antibiotics are available and there are vaccines against the main agents that cause **bacterial meningitis**, this disease still is an important cause of mortality and long-lasting neurological sequelae worldwide. It continues to be one of the 10 main causes of infant mortality in developed countries.

SPEED-OLIGO® BACTERIAL MENINGITIS (ref. SP006)

Under development products

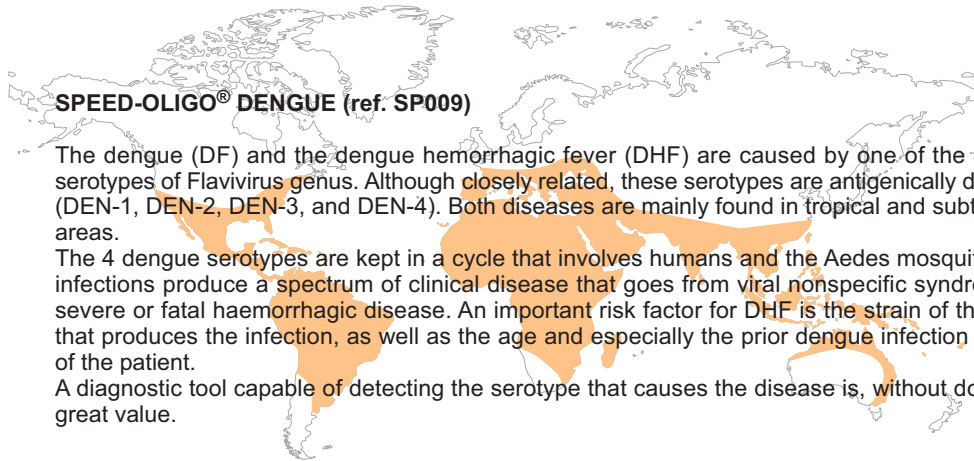
new references that increase Speed-oligo® panel

SPEED-OLIGO® RSV & hMPV (ref. SP010)

Acute respiratory infections (ARI) are the first cause of mortality and one of the main reasons of infant mortality worldwide. Respiratory syncytial virus (RSV) is one of the more relevant pathogens in ARI infections, and human metapneumovirus (hMPV) is a recently discovered pathogen, genetically related to human respiratory syncytial virus that causes respiratory tract infections and flu-like illness.

RSV and hMPV infections are present in all age groups (children, elderly people and immunocompromised patients) but predominate among infants younger than 5 years old. The available literature indicates that more than 70% of one-year old infants have been infected by RSV and that the rest will be infected in their second year of life. Every year, during the winter months, RSV is one of the main causes of consultation and hospitalization in health care centres due to epidemic outbreaks in breast-fed babies. Most of the hMPV infections take place in winter months too, and are widely diagnosed in children presenting pneumonia, bronchiolitis, bronchial asthma exacerbation, croup, and upper respiratory tract infection.

The conventional techniques for the diagnosis of RSV (in which two strains, A and B, are recognized) and hMPV turn to be laborious and variable. These difficulties in the diagnosis are partially responsible for the unknown aetiology of more than 50% of the ARI cases and thus prevent the adequate treatment of the disease. Rapid diagnostic techniques based on nucleic acid amplification are becoming increasingly necessary for RSV and hMPV detection.



SPEED-OLIGO® DENGUE (ref. SP009)

The dengue (DF) and the dengue hemorrhagic fever (DHF) are caused by one of the 4 virus serotypes of Flavivirus genus. Although closely related, these serotypes are antigenically different (DEN-1, DEN-2, DEN-3, and DEN-4). Both diseases are mainly found in tropical and subtropical areas.

The 4 dengue serotypes are kept in a cycle that involves humans and the Aedes mosquito. The infections produce a spectrum of clinical disease that goes from viral nonspecific syndrome to severe or fatal haemorrhagic disease. An important risk factor for DHF is the strain of the virus that produces the infection, as well as the age and especially the prior dengue infection history of the patient.

A diagnostic tool capable of detecting the serotype that causes the disease is, without doubt, of great value.

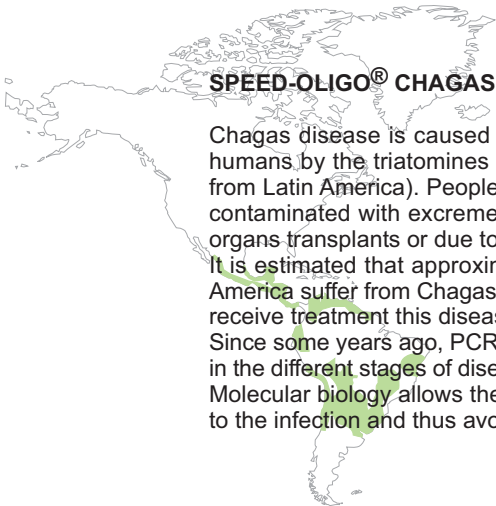
SPEED-OLIGO® ENTEROVIRUS (ref. SP007)

Acute infections due to enterovirus are the most common cause of aseptic meningitis. They mainly occur in summer and fall with great incidence in children.

Even if enterovirus infection could appear as an unspecific feverish disease, the virus is neurotropic and can affect the central nervous system. 50% of newborns and children with feverish disease caused by enterovirus have concomitant meningeal affection. The enterovirus can also cause severe septic syndromes in newborns such as meningoencephalitis, myocarditis and hepatitis, and have high morbidity and mortality in this type of population.

Traditionally, the isolation of enterovirus from cerebrospinal fluid (CSF) has been the selected diagnostic method. However, cell culture is rather laborious, time consuming and lacks sensibility. Furthermore, serological assays are not useful because of the serotype diversity of enterovirus. Due to all these problems, there are often difficulties to distinguish the disease caused by enterovirus from bacterial meningitis or sepsis, leading to the abuse of empiric intravenous antimicrobial therapy and lengthy hospitalizations.

Molecular amplification methods such as PCR offer new possibilities to improve the diagnosis.



SPEED-OLIGO® CHAGAS (ref. SP012)

Chagas disease is caused by the parasite *Trypanosoma cruzi*. It is transmitted to animal and humans by the triatomines insect vectors that are only found in America (mainly in rural areas from Latin America). People can also be infected by the consumption of raw food that has been contaminated with excrement of insects infected, congenital transmission, blood transmission, organs transplants or due to accidental exposure in labs.

It is estimated that approximately 8 to 11 million people in Mexico, Central America and South America suffer from Chagas, most of them do not even know that they are infected. If they do not receive treatment this disease can last their entire lives and can be even mortal.

Since some years ago, PCR technique has been introduced as a parasitologic diagnostic method in the different stages of disease and it has completely replaced the classic parasitologic methods. Molecular biology allows the early detection in newborns even earlier than a serologic response to the infection and thus avoids false positives due to the inherited antibodies from the mother.

SPEED-OLIGO® TOXOPLASMA (ref. SP014)

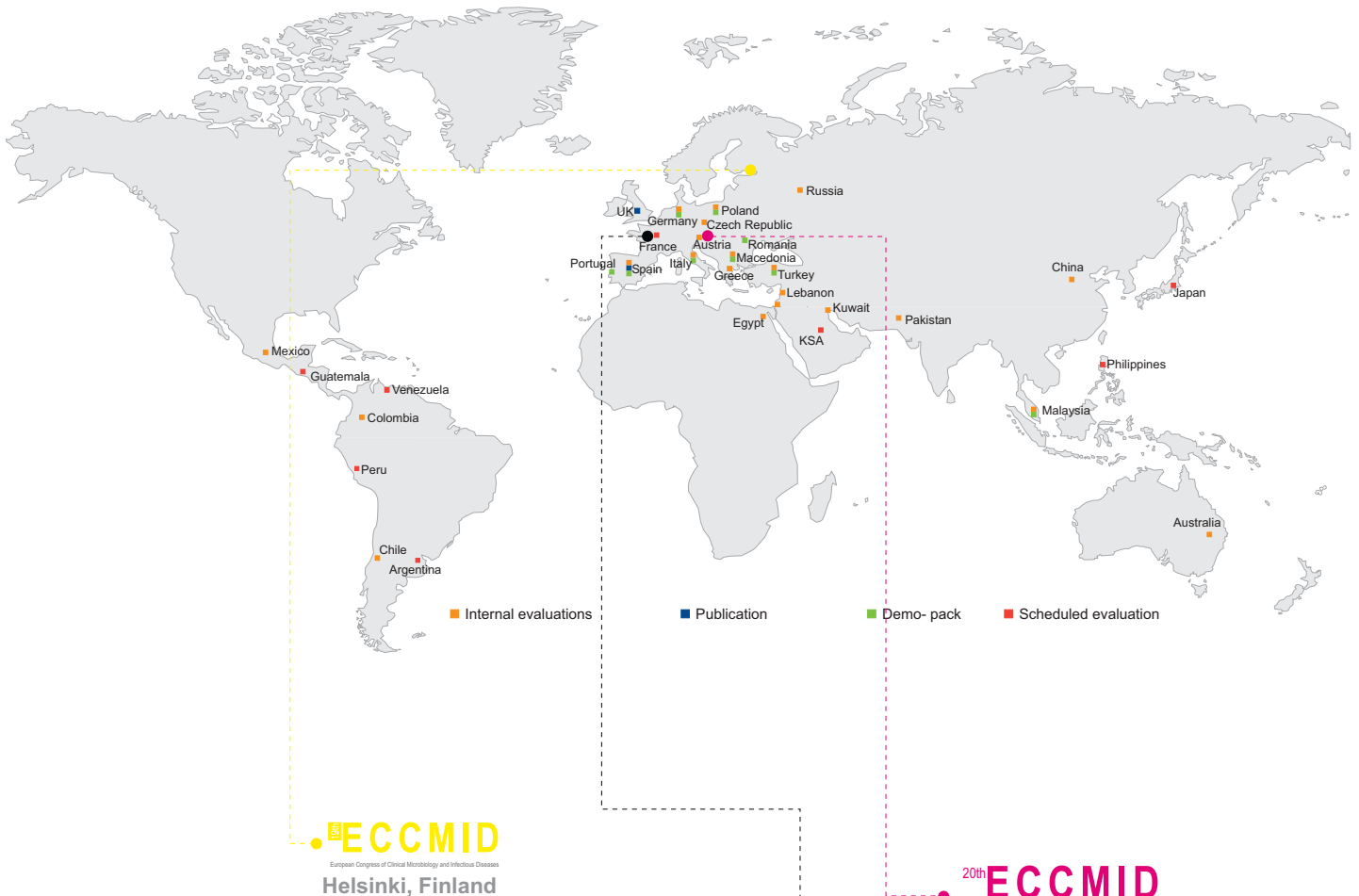
Toxoplasma gondii is an obligate intracellular protozoan parasite found worldwide. Most infections are benign although severe symptoms appear in immunocompromised patients or congenital infections. Women infected during the first quarter of pregnancy can have spontaneous abortion and the foetus can develop hydrocephalus.

Toxoplasmosis diagnosis is normally done by serological tests, although positive levels of IgM response detected up to a year after the infection make these techniques less useful.

PCR technique has been performed with success in the congenital and ocular toxoplasmosis and in the diagnosis of immunosuppressed patients. The most common use of PCR is the prenatal diagnosis of congenital infection by using amniotic fluid.

On-going evaluations

worldwide presentation in the most important centres interested in new technologies



9th ECCMID
European Congress of Clinical Microbiology and Infectious Diseases
Helsinki, Finland

Rapid oligochromatographic assay for the detection of GBS (group B streptococcus) in vaginal samples

Carrillo, J.A., Roldán, C., Gassó, J., Mendoza, J., Rojas, J., Camacho, A.

Evaluation of an oligochromatographic test for identification of mycobacteria most frequently isolated in human from liquid and solid culture media

Martínez-Lirola, M.J., Montiel Quezel-Guerraz, N., Marín Arriaza, M., Carrillo, J.A.

Evaluation of two real-time PCR methods to direct detection of group B Streptococci against conventional chromogenic culture

Jesús de la Calle, I., Roman-Enry, M., Rodríguez- Iglesias, M.A.

20th ECCMID
European Congress of Clinical Microbiology and Infectious Diseases
Vienna, Austria

Preliminary evaluation of a rapid oligochromatographic assay for the detection of Bordetella species in respiratory samples

Carrillo, J.A., Pedrosa-Corral, M., Pérez-Ruiz, M., Mendoza, J., Rojas, A., González, D., Navarro-Marí, J.M.



Evaluation of an oligochromatographic test for Legionella pneumophila detection in respiratory samples

Lindsay, D., Carrillo, J.A., Rodríguez-de la Rosa, I., Brown, A.



Pérez-Ruiz M., Navarro-Marí J.M., Bautista-Marín M.F., Pedrosa-Corral I., Sanbonmatsu-Gómez S., Camacho A. G., Rojas J.; Ruiz-Ortiz J., Rodríguez-Granger J. and Carrillo J.A. 2010. **Development and preliminary evaluation of a rapid oligochromatographic assay for specific detection of new human influenza A H1N1 Virus.** Journal of Clinical Microbiology: 1801-1805

Speed-oligo[®] products vs. other techniques

growing range of products with advantages against other techniques

SPEED-OLIGO[®] CHLAMYDOPHILA PNEUMONIAE (ref. SP003)

SPEED-OLIGO[®] MYCOPLASMA PNEUMONIAE (ref. SP001)

- Saving time and greater efficiency against traditional culture methods.
- Speed-oligo[®] offers a contemporary diagnosis versus the retrospective serological methods. Besides, it has to be taken into account that adult patients do not develop IgM antibodies.

SPEED-OLIGO[®] LEGIONELLA PNEUMOPHILA (ref. SP002)

- Speed-oligo[®] is a complementary test to antigenuria because it allows the detection of all serogroups and more than 25% of the patients present negative value with antigenuria.
- Against serology, Speed-oligo[®] offers a contemporary diagnosis instead of a retrospective view.

SPEED-OLIGO[®] BORDETELLA (ref. SP011)

- Speed-oligo[®] offers a faster and more sensitive diagnosis than culture.
- Against serology Speed-oligo[®] provides a contemporary diagnosis.

SPEED-OLIGO[®] MYCOBACTERIA (ref. SP005)

- Compared to biochemical methods, Speed-oligo[®] saves time and provides simplicity.
- Speed-oligo[®] is faster and its technique is more accessible than other hybridization methods.

SPEED-OLIGO[®] DIRECT MYCOBACTERIUM TUBERCULOSIS (ref. SP016)

- Against smear microscopy, Speed-oligo[®] enables the differentiation between Mtb complex and MOTT infections with better sensitivity and specificity, thus improving patient management.
- Against culture, it greatly reduces the time required to get results and therefore allows to undertake the isolation measurements and start the contact investigation weeks in advance with regard to conventional diagnosis.

SPEED-OLIGO[®] BACTERIAL MENINGITIS (ref. SP006)

- Speed-oligo[®] is a faster method than traditional culture methods and is less sensitive to external factors such as antibiotics treatment or transport conditions.
- Against antigen detection and staining, Speed-oligo[®] is more sensitive and specific.

SPEED-OLIGO[®] GROUP B STREPTOCOCCUS (ref. SP004)

- Speed-oligo[®] is more sensitive than prenatal research and risk analysis.
- Speed-oligo[®] gives results before delivery in comparison with culture, which requires 24-48 hours.

SPEED-OLIGO[®] DENGUE (ref. SP009)

- Speed-oligo[®] offers a current diagnosis against the retrospective view provided by serology.
- Versus antigenemia, Speed-oligo[®] incorporates the advantage of identifying the type of Dengue virus.

SPEED-OLIGO[®] ENTEROVIRUS (ref. SP007)

- Speed-oligo[®] is more sensitive than culture and improves the availability of results.
- Compared to clinical and lab algorithms, Speed-oligo[®] improves the specificity.

SPEED-OLIGO[®] RSV & METAPNEUMOVIRUS (ref. SP010)

- Versus culture, Speed-oligo[®] offers rapid results and its technique is simpler.
- Contrary to the antigen detection techniques, it adds more sensitivity and specificity.
- Speed-oligo[®] offers a current diagnosis instead of retrospective such as the serological techniques. Besides, it has to be taken into account that adult patients do not develop IgM antibodies when a reinfection occurs.

SPEED-OLIGO[®] CHAGAS (ref. SP012)

- Speed-oligo[®] allows early detection of congenital disease, which is impossible with serology.
- Versus direct methods, Speed-oligo[®] is simpler and more specific (staining) and the technique is more simple (xenodiagnosis).

Key points of the assay procedure

conclusions after performing the protocol in extreme conditions

PCR Mix resuspension

EXTREME CONDITION	DESCRIPTION	CLASSIFICATION	RESULT
Variation in the resuspension volume	± 25 %	Minor	Little changes in signal intensity
Shake mix (10")	Shaken- non shaken	Very important	Non shaken mix does not amplify efficiently
Reconstitution time	Non incubation and various incubation times	Important	Short and long incubation times reduce amplification intensity

CONCLUSIONS

- Volume variations do not affect results noticeably.
- Vortex is important for a homogeneous mixture.
- Dissolving needs time, but incubation excess may affect reagents.

Thermocycler

EXTREME CONDITION	DESCRIPTION	CLASSIFICATION	RESULT
Annealing temperature	55°C ± 2°C	Very important	Different temperature leads to inefficient amplification
Denature, annealing and extension times	15"-15"-15"	Very important	Less than 20" leads to inefficient or no amplification

CONCLUSIONS

- Primers are designed for a specific annealing temperature.
- Taq polymerase needs a minimum time to amplify.

Samples

EXTREME CONDITION	DESCRIPTION	CLASSIFICATION	RESULT
Extraction	Sample used directly	Very important	Samples may contain inhibitors
Volume variation in PCR	± 50%	Very important	Variations leads to inefficient amplification

CONCLUSIONS

- Samples must be extracted as indicated to avoid inhibitors and low DNA concentrations.
- Variations in PCR mix affect reagents concentrations.

Running buffer

EXTREME CONDITION	DESCRIPTION	CLASSIFICATION	RESULT
Volume variation	± 35 %	Minor	Little signal variation
Temperature	55°C ± 5°C	Very important	Temperature variation leads to inefficient hybridization

CONCLUSIONS

- Running buffer is not a factor that varies results drastically.
- Probes are designed for a specific hybridization temperature. Use 1.5 ml microtubes.

Denaturing

EXTREME CONDITION	DESCRIPTION	CLASSIFICATION	RESULT
Temperature change	Slow- fast	Very important	Slow cooling leads to DNA strands to bind and to not hybridize with probes
Time between denaturation and hybridization	Long- short	Important	Long waiting time leads to DNA strands to bind and to not hybridize with probes

CONCLUSIONS

- Use ice block, crushed ice or put sample immediately from thermocycler into the tube.
- Hybridize in less than 1'. Denature again if necessary. Have thermocycler and thermoblock in the same area.

Hybridization

EXTREME CONDITION	DESCRIPTION	CLASSIFICATION	RESULT
PCR product volume	± 50%	Important	Less volume leads to signal intensity reduction
Time reading	Soon and later	Very important	False positive results may appear if strips are read with delay

CONCLUSIONS

- Adding right PCR product volume is important for correct detection and reading.
- Reading the strips in the following 5 minutes after hybridization is necessary.

Troubleshooting guide

No signal in any line

POSSIBLE CAUSE	SOLUTION
Damaged/ruined strips	Check storage (dry and 2-8°C) and strip usage. Control product reception.
Insufficient running buffer volume	Check if volume is correct and if running solution reaches the top of the membrane.
Strip does not reach the bottom of the tube during the hybridization	Use 1.5 ml microcentrifuge tubes as recommended.

No signal in amplification control and sample lines

POSSIBLE CAUSE	SOLUTION
PCR mix might not be prepared correctly	Check that mix has been resuspended in right volume, shaken and incubated as recommended.
Denaturing might not be done correctly	Check if volume is correct and if running solution reaches the top of the membrane.
The PCR product volume used might be insufficient	Check volumes used and pipettes.
Wrong thermocycler program or thermocycler malfunction	Check program used and thermocycler function.
Thermoblock with wrong temperature	Check thermoblock temperature.
Ruined PCR mix	Check that the procedure has been done correctly. Check storage (2-8°C unopened).

No signal in positive control

POSSIBLE CAUSE	SOLUTION
Positive control might be ruined	Check storage (dry place at 2-8°C if lyophilized and minus 20-70°C once resuspended)

Controls and control lines with signal but no signal in samples lines

POSSIBLE CAUSE	SOLUTION
Sample extraction might not be done correctly	Spike samples with DNA control or serial diluted DNA samples.
Sample volume might be below detection limit	Elute extraction in smaller volume or as advised in extraction kit protocol.
Sample and sample extraction conservation	Conserve samples and sample extractions at -20°C or at -80°C. 'No frost' refrigerators are not recommended for sample conservation.

Signal in the negative control test line or in all the strips

POSSIBLE CAUSE	SOLUTION
Possible background noise	Blow dry strips immediately after hybridization.
The denaturing might have been done incorrectly	Minimize time between denaturing and hybridization and use ice blocks, crushed ice or put sample directly from thermocycler into the tube.
Possible PCR contaminated	Check and clean pipettes, rooms, surfaces, etc. with 5% bleach or commercial products (i.e. DNAzap). Have preamplification and postamplification procedures kept in different rooms.

Frequently Asked Questions

1. What kind of clients can use this new line of products?

Speed-oligo[®] is designed to bring Molecular Biology techniques into laboratories of diagnosis of infectious diseases through a very simple technique. Speed-oligo[®] combines all the advantages of Molecular Biology in a quick detection.

Any laboratory with minimal equipment can introduce the PCR technique, benefiting from the high reproducibility, sensitivity and specificity provided by the Speed-oligo[®] commercial kit without forgetting the advantages of chromatography revealing.

Even clients utilizing RT-PCR may be interested in Speed-oligo[®]. Vircell offers “exotic” parameters that require a low number of determinations, making Speed-oligo[®] more interesting than RT-PCR in terms of price and equipments expiration.

2. What are the equipment requirements to carry out the Speed-oligo[®] technique?

It is possible to carry out the Speed-oligo[®] technique with just a thermocycler (conventional or fast) and a thermoblock. These instruments are very common in Molecular Biology laboratories and do not need to be used exclusively for this technique.

3. What kind of samples are used in Speed-oligo[®]?

As with any other PCR technique, Speed-oligo[®] is assayed with nucleic acid. Each Speed-oligo[®] reference needs a different type of sample from which the nucleic acid is extracted. For some of the kits respiratory samples are used (Bacterial pneumonia combi, Influenza A H1N1, *Mycobacterium tuberculosis*, *Chlamydomphila pneumoniae*, *Legionella pneumophila*, *Bordetella* or *Mycoplasma pneumoniae*) while cerebrospinal fluid (Bacterial meningitis), vaginal or rectovaginal samples (Group B streptococcus) or culture (Mycobacteria) are used for other kits.

4. It is necessary to extract the nucleic acid from the samples in order to conduct the Speed-oligo[®] technique. Are reagents included in the kits to extract the samples?

Regarding samples extraction, Vircell offers reagents in Group B streptococcus and in Mycobacteria determinations. In these two references VIRCELL SAMPLE SOLUTION is included, which allows high quality DNA to be obtained to amplify with PCR by heating the SOLUTION with the sample to 95°C. Nevertheless, it is not possible to use this extraction method with other Speed-oligo[®] references since cerebrospinal fluid and respiratory samples contain a high amount of inhibitors that have to be eliminated by using a specific extraction kit. In these references any manual or automated commercial kit can be used (Qiagen, Roche, etc.).

5. May sample extraction be conducted with a classic extraction kit?

Speed-oligo[®] has been tested with different extraction kits: High pure PCR template preparation kit (Roche), QIAamp DNA blood mini kit (Qiagen) or NucleoSpin tissue (Macherey-Nagel). However, other commercial kits can be used. It must also be taken into consideration that the references for Group B streptococcus and Mycobacteria include the reagent VIRCELL SAMPLE SOLUTION to extract DNA.

6. This method is easy to conduct and the results can be seen very clearly, but for some clients contamination risk may be a disadvantage. What are the contamination risks when using Speed-oligo[®]?

The PCR mix included in the kit is prepared and contains all the necessary components, even Taq polymerase. As a consequence, manipulation and pipetting are minimal and the contamination risk is, therefore, dramatically reduced. A prepared PCR mix in several vials (it is only necessary to resuspend and aliquot) is an innovation in comparison with other PCR or qPCR kits.

Any PCR technique, even qPCR, may present contamination risk from controls or samples. Provided that the basic working norms in a laboratory are followed, there should not be any contamination problem.

In a normal PCR laboratory there should be, as a minimum requirement, a preamplification area where the reaction mix is prepared in, a PCR cabinet and a postamplification area where samples are manipulated and results are revealed. Following these recommendations should prevent any contamination problem with Speed-oligo[®].

7. Can I conduct Speed-oligo[®] in my usual laboratory?

We recommend using this technique in a Molecular Biology laboratory with well differentiated pre- and post-amplification areas.

In our effort to help clients, Vircell includes in this document a practical guide to setting up a PCR laboratory in accordance with international standards.

8. In some countries, Molecular Biology laboratories are highly sensitive to prices. What is Speed-oligo®'s market price?

Considering the distribution channel necessary for this kind of product, Vircell has established a very competitive price per strip as the target price. Even though Speed-oligo® is a technologically advanced product, its price policy is to position itself as a Molecular Biology technique affordable for any kind of laboratory. In the price analysis made before launching the product onto the market, the price of Speed-oligo® turned out to be 2 to 4 times lower than qPCR and other similar techniques on the market, and a bit higher than conventional PCR. Thus, Speed-oligo® offers the reproducibility advantages of a ready-to-use commercial kit and it is very simple to use as well.

9. What is the expiry date of Speed-oligo® product line?

All Speed-oligo® products have a shelf life of 12 months.

10. What are the transport conditions of Speed-oligo®? Does it require the use of dry ice?

Speed-oligo® does not require special conditions for transport. As this product includes the positive control and the PCR-mix in lyophilized presentation the transport can be done at room temperature. The storage should be done refrigerated at temperatures between 2 and 8°C.

11. After the final step of PCR denaturation and before the hybridization of the strips, is it necessary to place the samples on ice?

Speed-oligo® is a very easy technique but the assay protocol has some critical procedures that need to be strictly followed as the instructions indicate:

- It is important that strip detection takes place no longer than one minute after denaturation. If the PCR product is not added to the 1.5 ml tubes immediately after the final denaturation step of the PCR programme, then it will be necessary to repeat this step once again (95°C for 1 minute).
- The thermoblock has to be preheated to 55°C and the hybridization solution should be incubated for two minutes at that temperature.
- After the final denaturation step of the PCR programme the samples have to be transferred to the thermoblock by using an isofreeze tube rack or a normal test tube rack on ice. A test tube rack at room temperature should never be used.
Another alternative is pipetting the sample without cooling just after denaturation at 95°C and introducing the strip immediately. For the strip detection it is necessary that the DNA is denatured (single strand). If the samples are at room temperature, denatured DNA rehybridizes and then it is not possible for the hybridization with the strip detection probes to take place.

12. How is it recommended to store Speed-oligo® results?

Provided that the client wants to store the revealing strips, we recommend the use of the archive sheets included in the kit. This should take place in the normal working laboratory (postamplification area), but never in the PCR laboratory to avoid contamination. Another possibility would be to scan or photocopy the results and eliminate the revealing strips.

13. Is it possible to use SPEED-OLIGO® LEGIONELLA PNEUMOPHILA (SP002) with urine samples?

Our reference SP002 has only been assayed with respiratory samples and not with urine. Nevertheless, it has to be considered that urine samples contain many inhibitory substances of PCR and as a consequence, a very good extraction system should be utilized. The PCR amplification control line is a good system to control the presence of possible inhibitory substances in the sample. It also has to be taken into account that our reference SP002 is produced for an oligonucleotide present in all Legionella pneumophila serogroups but not in other legionella species. Several publications about the low sensitivity of the PCR technique in urine samples can be found in scientific literature. This sensitivity improves if the samples are collected during the first days of the infection.

14. SPEED-OLIGO® MYCOBACTERIA (SP005) is designed for mycobacteria identification in culture samples, but can it also be used directly with clinical samples?

Speed-oligo® has only been validated for identification in culture samples. Culture remains the reference technique in mycobacteria laboratories. Moreover, identification in positive cultures continues to be a challenge due to the fact that the existing methods are slow and require high technology. Speed-oligo® is simple and quick and it is consequently the perfect complement for mycobacteria culture because it allows differentiation between MTB and MOTT and the identification of MOTT in the same strip as well.

On the other hand, Vircell has recently developed the test SPEED-OLIGO® DIRECT MYCOBACTERIUM TUBERCULOSIS for the differential identification in clinical samples of members of the MTB complex and also any mycobacteria present, through the appearance of two specific lines in the dipstick. Additionally, an amplification control for a human gene allows the detection of any inhibitor present in the sample.

15. In cases where Speed-oligo® is used to identify mycobacteria, how much sensitivity does the equipment have? Is there a relevant publication available?

As stated in the instructions for use included in the kit, it has 98% sensitivity (100% in MTB) with 100% specificity. In the 19th edition of ECCMID- European Congress of Clinical Microbiology and Infectious Diseases- that took place in Helsinki, the following work was presented: *Evaluation of an oligochromatographic test for identification of mycobacteria most frequently isolated in human from liquid and solid culture media* (Martinez- Lirola, M.J. et al). This publication is available on our web page and can be also sent upon request.

16. Speed-oligo® versus sequencing.

Speed-oligo® does not allow an identification as exhaustive as sequencing. However, the advantage that Speed-oligo® can provide is based on the cost/benefit relationship.

17. Regarding SPEED-OLIGO® NOVEL INFLUENZA A H1N1 (Ref. SP015), which previous steps are necessary? Are all the reagents included in the kit?

Speed-oligo® includes all the reagents to perform the PCR reaction and the detection with strips. Prior to Speed-oligo® for nH1N1 diagnosis, it is necessary to use a RNA extraction kit together with a reversotranscriptase kit. In the product insert we recommend using the following kits, although similar products would be also valid: *QIAamp viral RNA* (QIAGEN) for RNA extraction and *iScript cDNA Synthesis kit* (Biorad) or *AffinityScript Multiple temperature Reverse Transcriptase* (Stratagene) for the reversotranscription.

18. Is there any scientific literature that supports the performance of SPEED-OLIGO® NOVEL INFLUENZA A H1N1 (Ref. SP015)?

A performance study of 253 samples done in collaboration with the Virgen de las Nieves University Hospital of Granada, showed 100% of specificity and 97.5% of sensitivity. This performance study, available in our website, has been recently published in the scientific magazine *Journal of Clinical Microbiology* (May 2010). Should you have any further questions or concerns, please do not hesitate to contact our Customer Service department at customerservice@vircell.com.

19. Is SPEED-OLIGO® NOVEL INFLUENZA A H1N1 able to detect different strains of flu?

SPEED-OLIGO® NOVEL INFLUENZA A H1N1 (2nd generation), besides the specific test line for Influenza A nH1N1, includes an additional test line in the dipstick, which is specific for generic Influenza A. Therefore, this product is able to discriminate between seasonal and nH1N1 flu. Furthermore, this new generation includes an improved amplification control line that, apart from checking the correct operation of the PCR mix and the absence of inhibitors, is able to confirm the quality of the genetic material in the sample by means of amplification of human **b** globulin gene.

20. Why does SPEED-OLIGO® BORDETELLA include two test lines?

In the new product SPEED-OLIGO® BORDETELLA, a test line to detect the presence of the insertion sequence *IS481* is included to maximize the sensitivity of the test, since *IS481* is present in multiple copies per genome. As *IS481*-like elements have been reported in other *Bordetella* species, a second test line to detect the presence of the specific pertussis toxin promoter (*ptxA-Pr*) gene has also been included.

Support tools

for the commercialization of this new product line

Since its first launching at MEDICA 2008 Exhibition, our Sales, Marketing and Technical teams have been actively introducing this product line to our distribution network. Our customers have interest in this new product concept, its technical fundamentals and the commercial strategy; most countries have already implemented it with great success.

For the time being, Vircell is keenly working in:

On going evaluations

Speed-oligo® is being tested in some big labs in Europe, South America, Middle East, and Asia-Pacific region. In some cases, formal articles will be published demonstrating the strength of this new technique and will help to reinforce the brand awareness. Already published articles are available upon request.

In-house Workshops at Vircell facilities

Our distribution network needs a deep knowledge of this technology in and out the lab. With this objective we have held two training seminars in our facilities (Granada, Spain):

- I Workshop (November 2008): Portugal, Turkey, Czech Republic, and France.
- II Workshop (February 2009): United Kingdom, Poland, Romania and Germany.
- III Workshop (April 2010): Mexico, Colombia and Uruguay.

During these occasions our partners received a theoretical training on Speed-oligo® followed by a practical demonstration in our Molecular Biology labs and an exhaustive analysis of the "FAQ". Finally, some commercial proposals were raised to the assistants, to carry out successfully the introduction of this innovative technique in the market.

Speed-oligo® presence at international congresses

Several Vircell partners have already introduced this product line in their most relevant local congresses. Pictures of these events are available in our website.

Latin America on-site Workshops

To fulfil our Latin America distributors' demand, Vircell have organized a Speed-oligo® workshop tour for Mercosur and The Andean Community. With the invaluable collaboration of our partners in Argentina and Colombia, Vircell gathered 42 people from 10 different distributors. In parallel, technical presentations were done to final customers of Buenos Aires and Bogotá with great success.

Customized trainings

Other end-users workshops have been scheduled on demand: Italy (April 2009), Germany (June 2009), Portugal (October 2009), Mexico (October 2009), Romania (January 2010), Malaysia (May 2010), Turkey (May 2010) and France (to be determined)

Speed-oligo® commercialization is a main objective of our strategic plan and we are open to comments and suggestions from our commercial network.

Complementary documentation

Other documents as Instructions for use, Bibliographic references, Promotional material, Commercial offer, Recommended instruments, Demonstration video, Extraction methods and Protocol for setting up a PCR laboratory are available at www.vircell.com.

INFORMATIVE DOSSIER

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Ready-to-use kits for the diagnosis
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