MULTIANÁLISIS BASADO EN CAPTURA MAGNÉTICA DE *Legionella pneumophila*.

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MÉTODO DE ENSAYO
APROPIADO AL
USO PREVISTO

ENAC
Nota Técnica 32

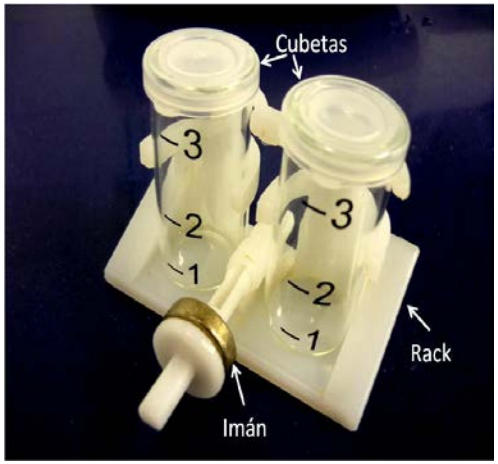
VALIDACIÓN/VERIFICACIÓN

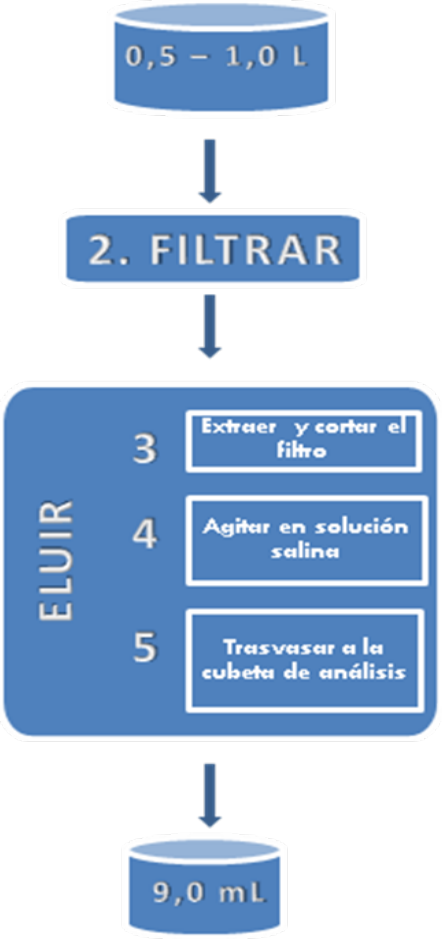
Muestras Reales (in vivo)

Muestras Artificiales (in vitro)

IPROma laboratorio

biótica® FAST DETECTION FOR LIFE

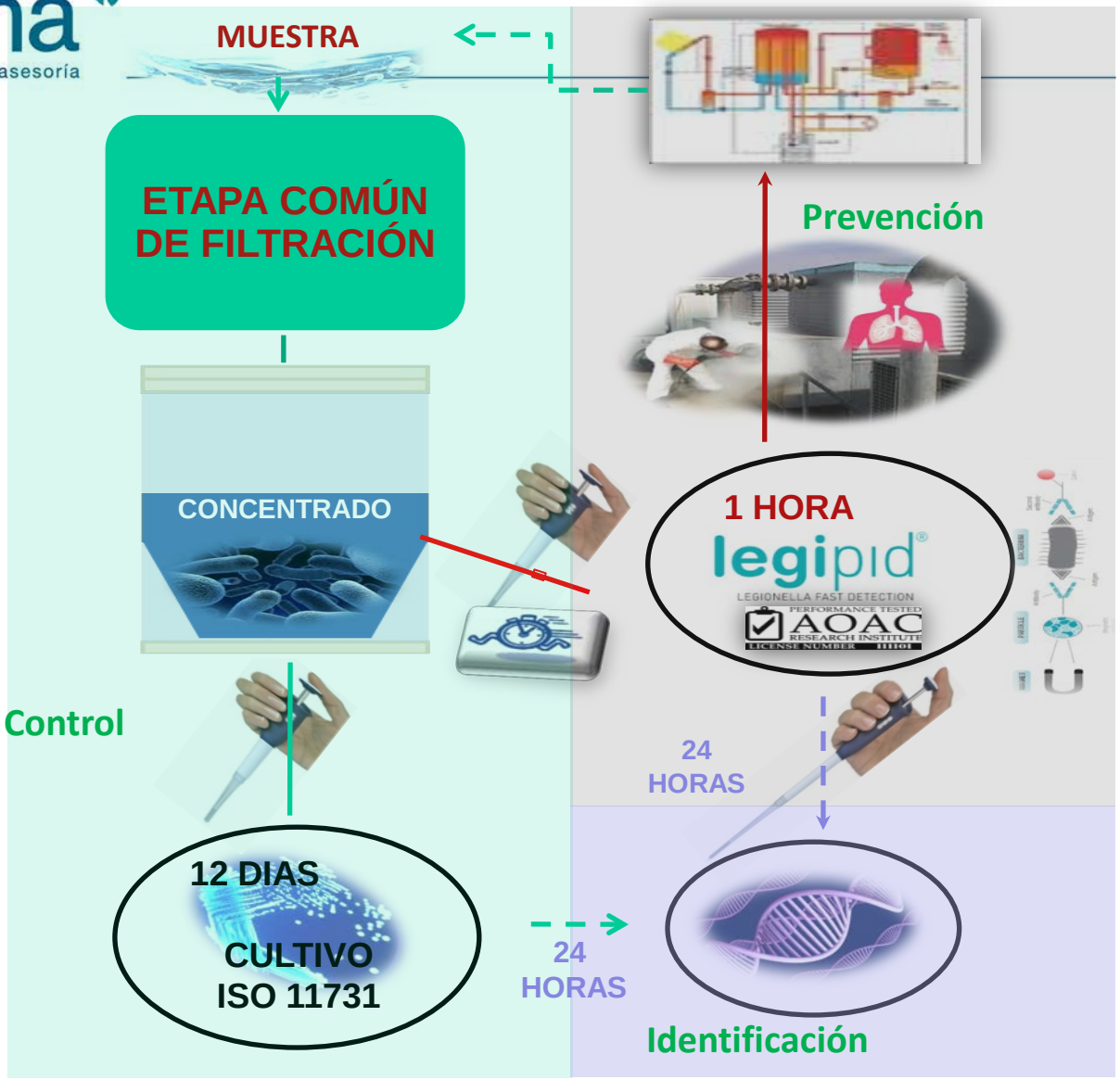


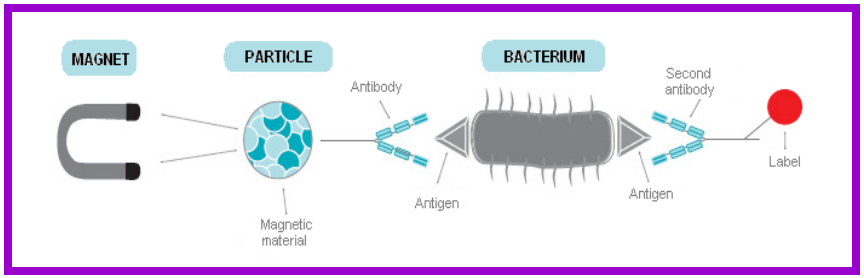


- Año 2009. Participación en la validación.

- Año 2010. Participación en el colaborativo. Acreditación por ENAC en nuestro laboratorio de un método basado en Legipid®.

- Año 2011- Certificación AOAC.


MUESTRA
ETAPA COMÚN DE FILTRACIÓN
CONCENTRADO
Prevención
1 HORA
legipid
LEGIONELLA FAST DETECTION
AOAC
Control
24 HORAS
12 DIAS
CULTIVO ISO 11731
24 HORAS
Identificación

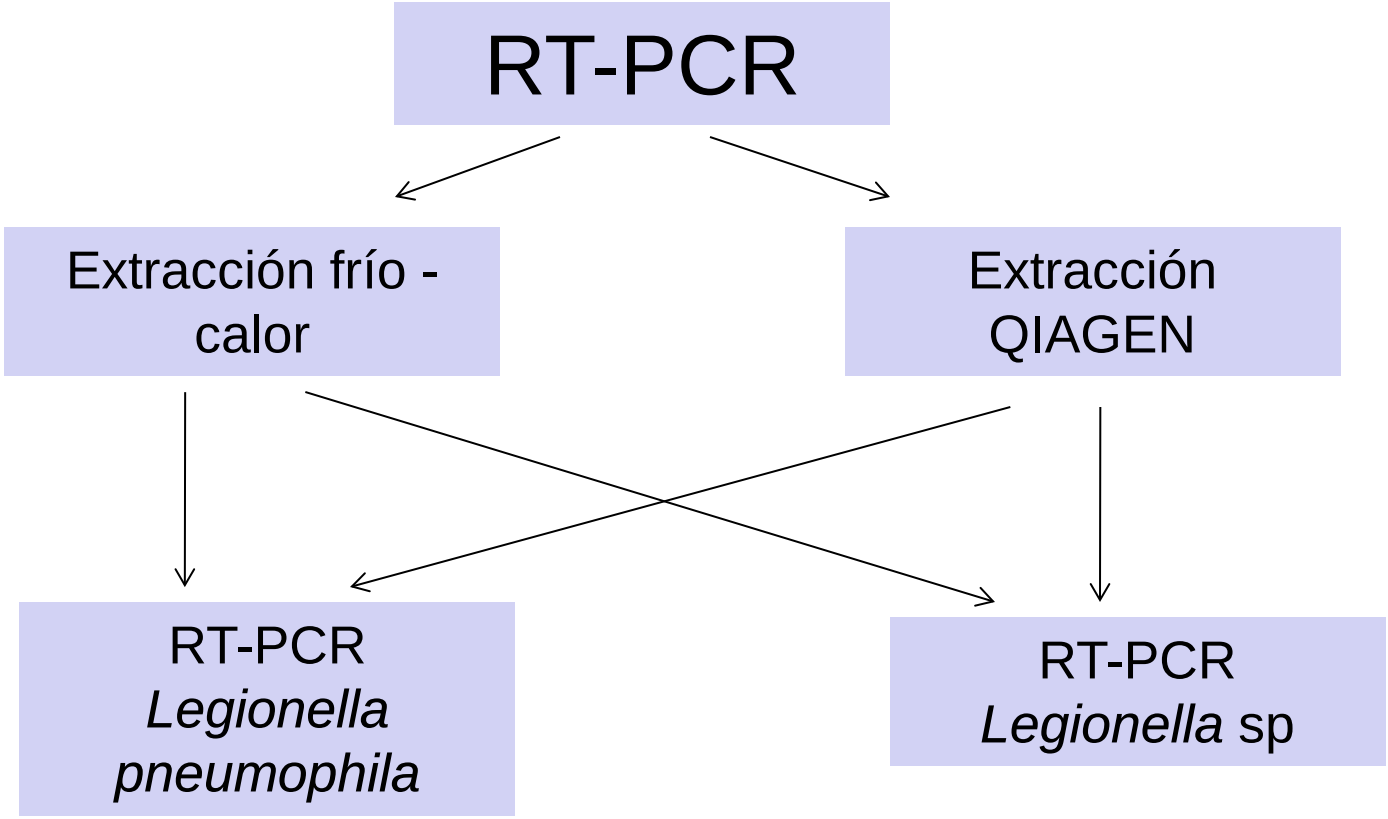


Captura inmunomagnética

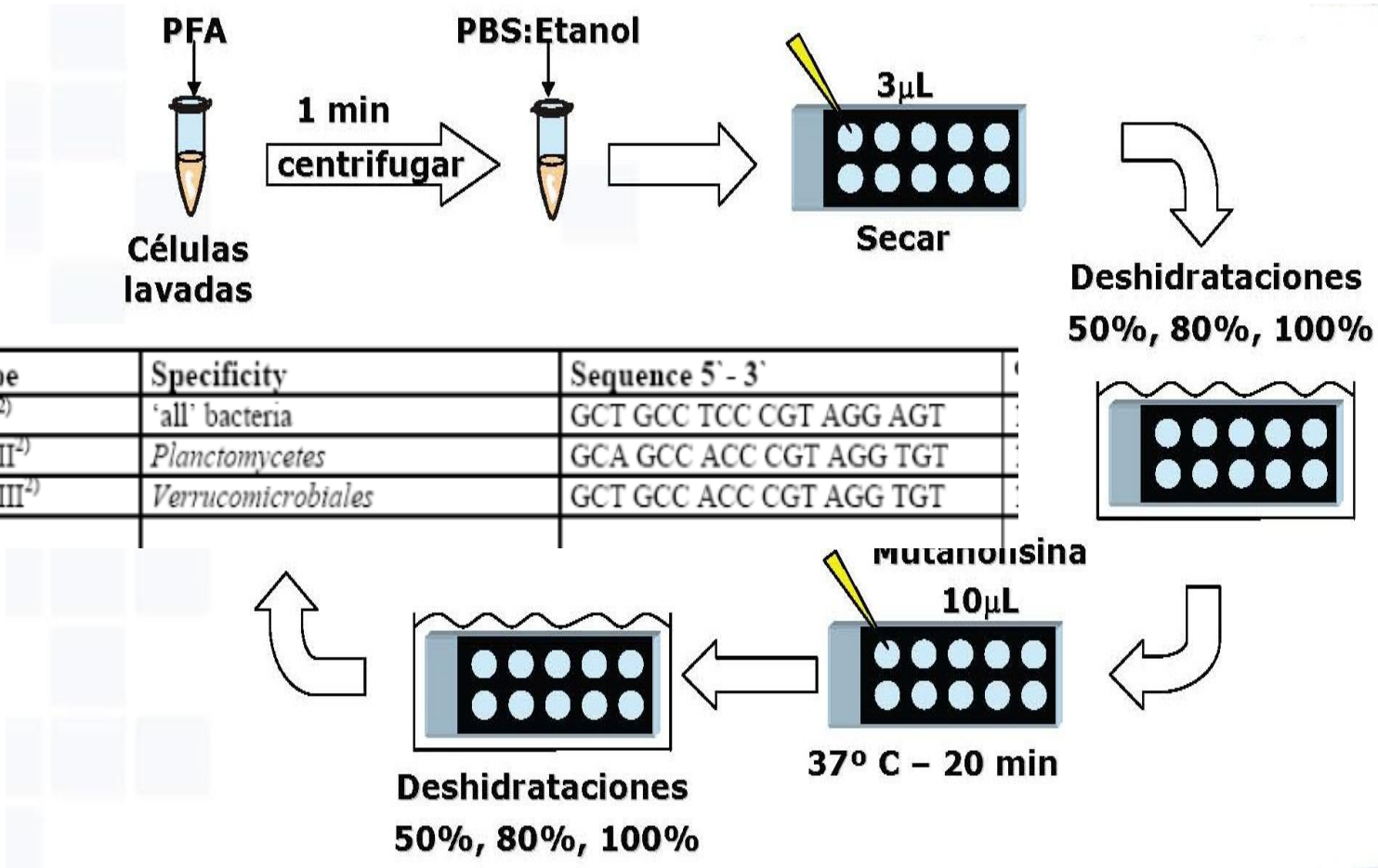
“biopellet”

RT-PCR

FISH

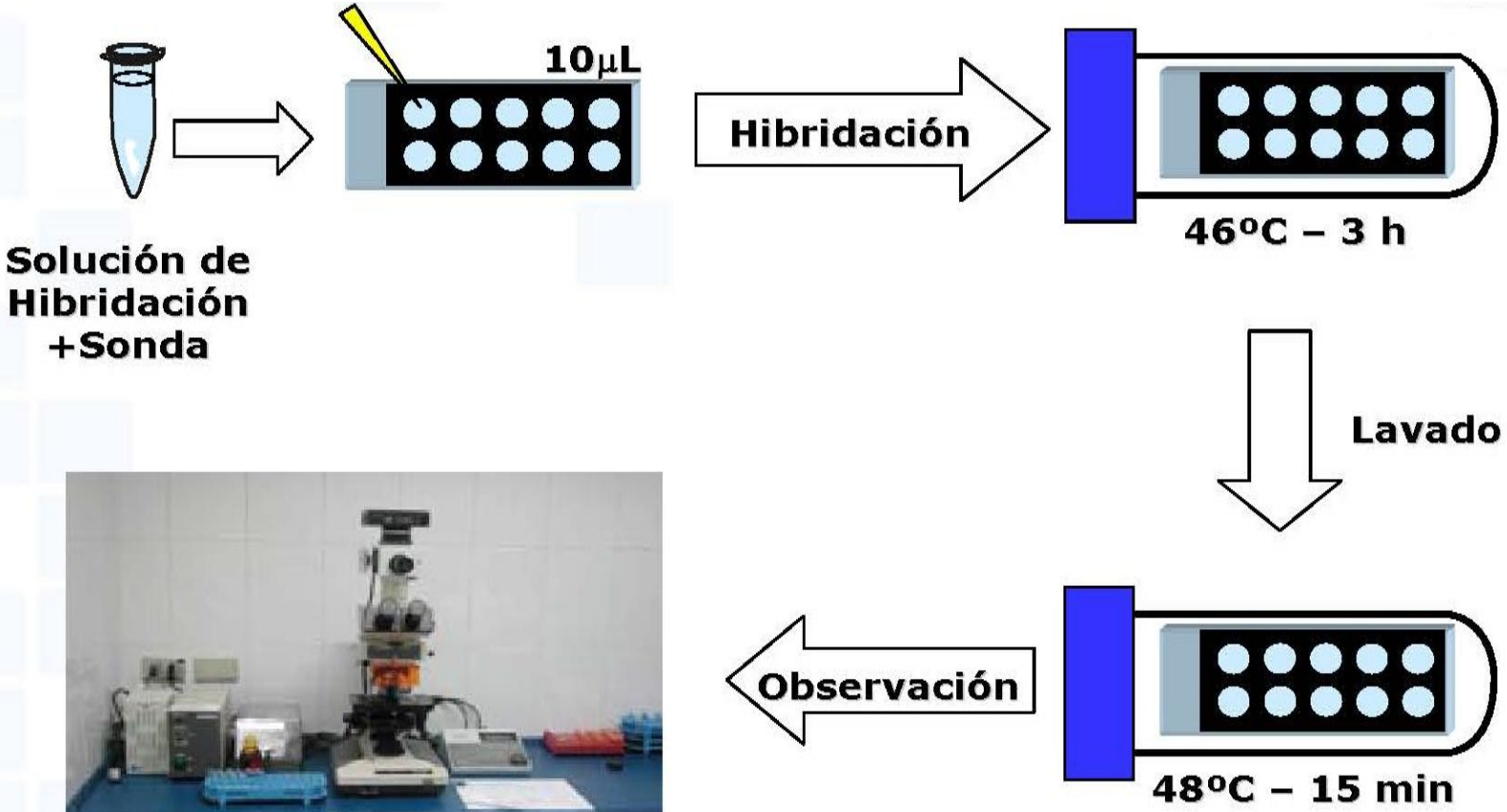

RT-PCR
Extracción frío - calor
Extracción QIAGEN
RT-PCR *Legionella pneumophila*
RT-PCR *Legionella* sp

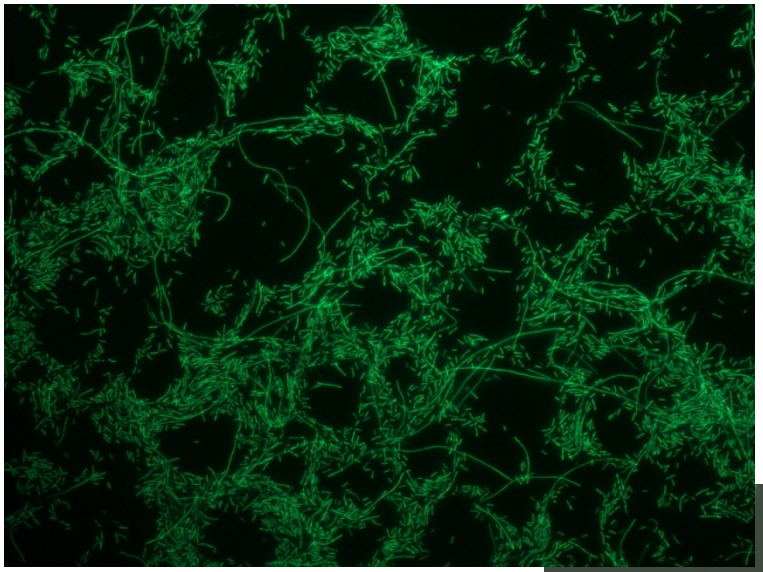
FISH

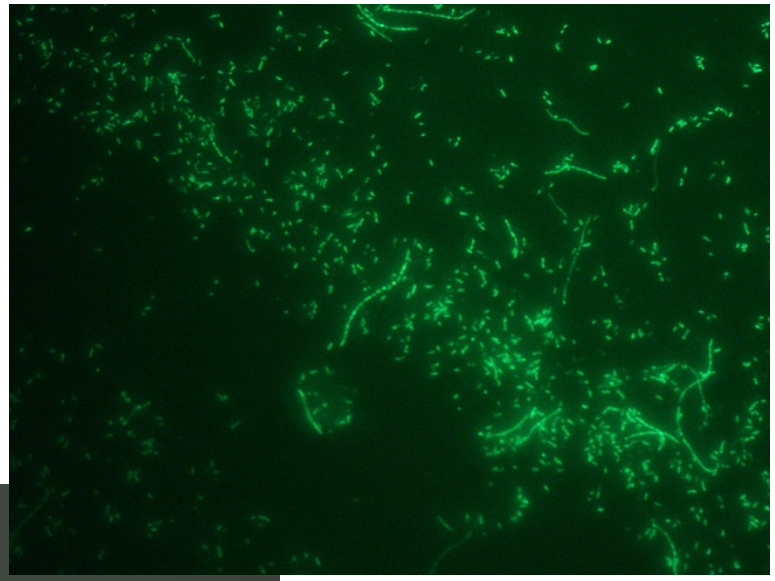


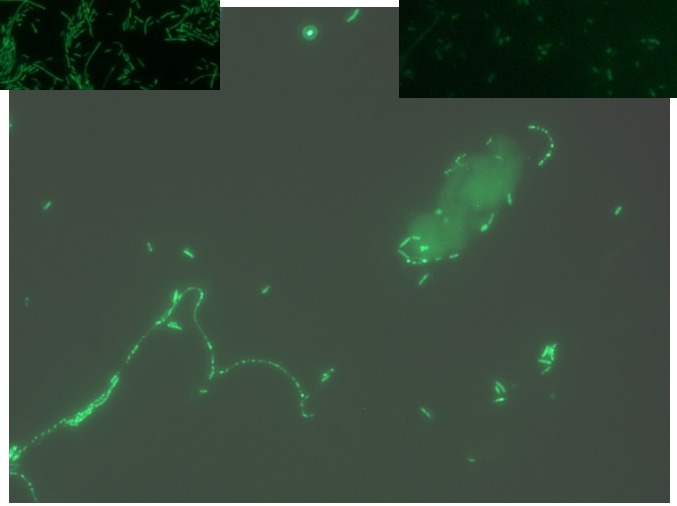
Probe	Specificity	Sequence 5`- 3`
EUB[2)]	'all' bacteria	GCT GCC TCC CGT AGG AGT
EUBII[2)]	*Planctomycetes*	GCA GCC ACC CGT AGG TGT
EUBIII[2)]	*Verrucomicrobiales*	GCT GCC ACC CGT AGG TGT

FISH

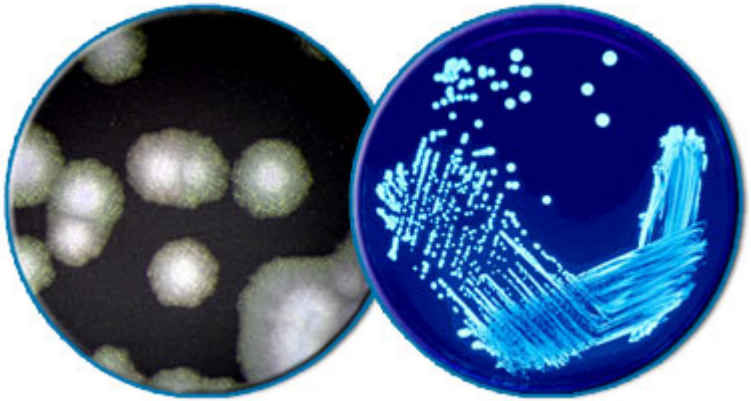

10µL
Solución de Hibridación +Sonda
Hibridación
46ºC – 3 h
Lavado
Observación
48ºC – 15 min

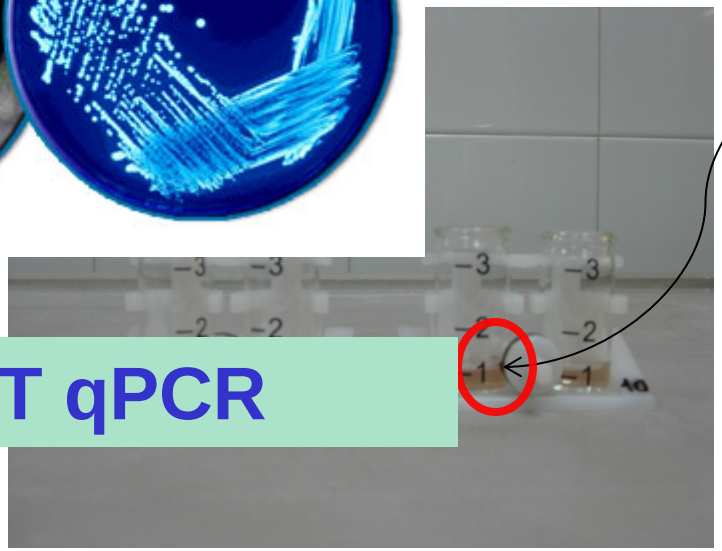






CEPA	PCR		FISH
	Legionella sp	*L.pneumophila*	MIX EUB
1358	+	+	+
1302	+	-	+
1368	+	+	+
1370	+	+	+
1318	+	+	+
1318	+	-	+
1333	+	-	+
1390	+	-	+
1393	+	-	+
1359	+	-	+
1358	+	+	+
1395	+	+	+
1369	+	+	+
1377	+	+	+
HPA	+	+	+





PMA – RT qPCR

Graci **FISH** a atenc

***Legionella* spp. (Manz et al. 1995):**
LEG705: CTG GTG TTC CTT CCG ATC
***Legionella pneumophila* (Grimm et al. 1998):**
LEGPNE1: ATC TGA CCG TCC CAG GTT