"PulseNet: Under the Microscope"
Volume 3

Alternative Agaroses – Results from External Validation and Recommendations

PulseNet's success relies on the ability to analyze and compare PFGE patterns generated in labs across the country. PulseNet laboratories must adhere to the instructions outlined in the standardized protocols to ensure that patterns are generated consistently within and between laboratories and comparable during analysis. Several improvements have been to the standardized protocols in recent years, but some key components have remained unchanged. One of these is the use of SeaKem Gold Agarose (Lonza) for the preparation of plugs and casting of the running gel. Recently, the PFGE Reference Lab at the CDC evaluated alternative types of agaroses. The evaluation focused on determining the effects on run time, normalization and overall quality of the gel image. Additional characteristics of the agaroses, including melting and solidifying times, gel strength and chemical properties were also noted. The impressions of each agarose, as well as results of the testing and recommendations for using each one, are given below. Overall, it was determined that laboratories following the standardized protocols generated acceptable results with two of the alternative agaroses for pouring gels and a third is being further tested. The Standardized Protocols will be updated to include the alternative agarose products. Feel free to contact **Molly Freeman** in the PFGE Reference Lab at **evy7@cdc.gov** with any questions.

Testing Alternative Agaroses for Use within the PulseNet Standardized Protocols

Background

- Until recently, SeaKem Gold (SKG, Lonza) was the only agarose validated for making plugs and running gels within PulseNet-standardized protocols due to its strength, superior resolution of bands and optimal run time.
- Other agaroses, including Certified Megabase (Bio-Rad) have been tested, but are **not** recommended due to low gel strength, fragment migration differences that lead to poor gel image normalization, and long electrophoresis run times.
- Bio-Rad recently developed a **new** formulation of Certified Megabase agarose and Amresco released a PFGE-grade agarose. IBI Scientific also distributes a PFGE-grade Agarose.

Approach

- Megabase and Agarose III™ were evaluated at the CDC as well as subjected to external validation by 5 state public health labs. State labs were asked to run certification strain sets.
- PulseNet-standardized protocols for each organism were followed **except** that 1% SKG (running gel) was replaced with either **1% Amresco Agarose III™ (LF or long fragment)** or **1% New Megabase**.
- Plugs were made from SKG, Amresco III™ or New Megabase agarose.
- Because of favorable initial results, external validation was extended to 11 labs for use while running routine isolates.

Agaroses Tested	Organisms Tested	Characteristics evaluated
Agarose III™ – Amresco®	*Campylobacter*	Appearance and handling characteristics of molten and solidified 1% agarose
Megabase – Bio-Rad	*E. coli O157 and non-O157*	Effect on plug preparation
PFGE Agarose – IBI Scientific	*Listeria monocytogenes*	Effect on run time
SeaKem Gold (Lonza)	*Salmonella enterica* spp	Effect on normalization
	Shigella sonnei	Cost comparison
	Vibrio cholera	

Evaluation

- **Labs:** Asked for feedback from PulseNet labs on their impressions of each agarose, whether used for making plugs or casting the running gel, and also collected information on source of TBE, instrument gel was run on, run time, gel length and normalization.
- **Database:** Of 118 gels run with either **new** Megabase or Agarose III™, 20 were selected and images submitted to database managers to be scrutinized. Managers were blinded to type of agarose. Feedback was obtained on band appearance (fuzzy or distinct), resolution, ghost bands, granularity / gel appearance, gel length, normalization (squished or stretched out bottom or top) and any other organism-specific differences.

Tables I and II. Summary of gels run for external validation. A total of 118 gels run on all organisms, except the *Vibrio* species, with the most being run with *Salmonella*, followed by *E. coli* O157 and *Shigella*. Twelve labs, including CDC and one international lab, ran 1 – 25 gels each. They were asked to run routine isolates with whichever agarose they chose. CDC provided validating labs with both Agarose III™ (Amresco) and **new** Megabase (Bio-Rad) for the validation. Labs were given the option to prepare plugs with either SKG or the same agarose used in the running gel. Most labs chose to make plugs with SKG. Tiffs were uploaded to the National Database and a copy was sent to the PFGE Reference Unit at CDC to keep track of what was run, and at the same time, compile a large dataset of gels and plugs made from various agarose products.

Table I. Gels run by organism

Organism	Agarose		Total
	Amresco	BioRad	
E. coli O157	8	9	17
non-O157	4	4	8
Campy	2	1	3
Listeria	6	3	9
Salmonella	34	36	70
Shigella	8	5	13
V. cholerae	0	0	0
Total =	62	58	120

Table II. Gels run by lab

Lab	Agarose		Total
	Amresco	BioRad	
CDC	7		7
ARG	1		1
CT	1	9	10
HI	3		3
ID	4	4	8
KS	1	1	2
MI	13		13
MN		13	13
NH	7	3	10
OK	14	11	25
SD	10	7	17
VA		9	9
Total =	61	57	118

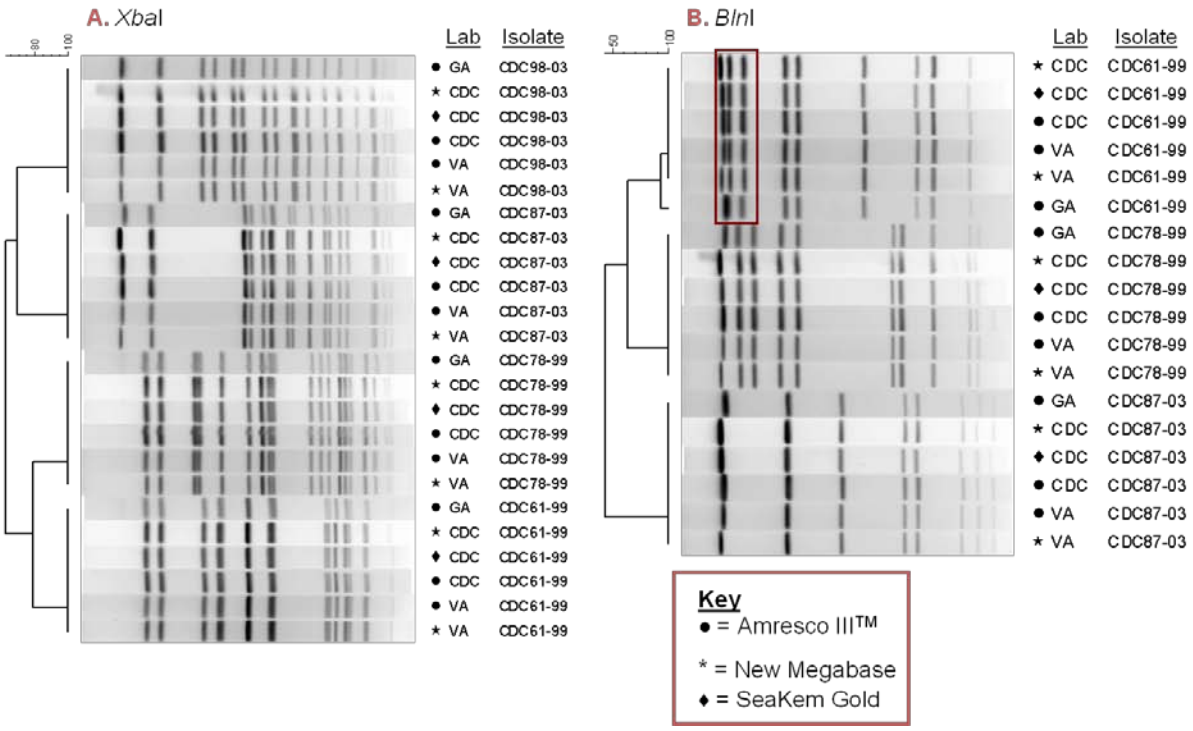
Table III. Comparison of characteristics of agaroses for running PFGE gels. By comparing important properties, such as gelling and melting range, gel strength, etc…, it is apparent that these agaroses have similar characteristics. Also, comparing each agarose to SeaKem Gold, our lab and collaborating labs reported a few differences. For example, most labs reported that the **new** Megabase took longer to melt and that solidified gels seemed stronger and took longer to run. Feedback from the labs was somewhat mixed for the Amresco agarose. For instance, some labs reported identical run times while others were longer.

Characteristics		Lonza SeaKem Gold	Amresco III™ (aka LF)	Old Bio-Rad Megabase	New Bio-Rad Megabase	IBI PFGE
Gelling Range (°C)		36 ± 1.5	37 - 41	36	36	36 ± 1.5
Melting Range (°C)		≥ 90	93 - 96	88	N / A	88 ± 1.5
Gel strength (g/cm^2)	1.0%	≥ 1,800	N / A	≥ 1,800	N / A	≥ 1800
	1.5%	≥ 3,500	≥ 2,000	≥ 3,200	≥ 3,200	≥ 3200
EEO (m_r)		≤ 0.05	0.06	≤ 0.12	≤12	≤ 0.12
Moisture (%)		N / A	8.5	< 7	N / A	< 7
Sulfate (%)		N / A	0.06	≤ 0.12	≤12	≤ 0.12
RNAse, DNAse, and Protease activity		None detected	None detected	None detected	None detected	None detected

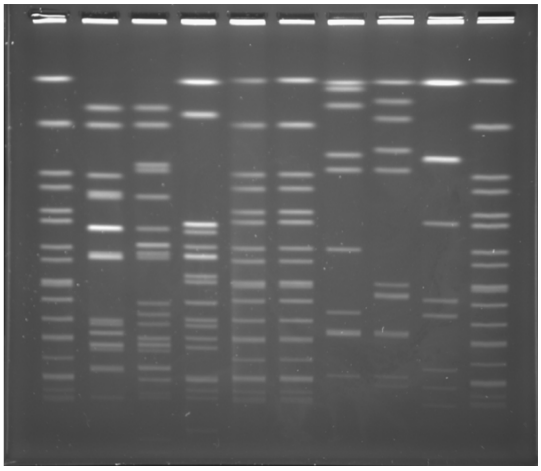
Observations in comparison to Lonza SeaKem Gold	Amresco III™ (aka LF)	Bio-Rad Megabase	New Bio-Rad Megabase	IBI PFGE
Melting time	+ / -	+ / -	+	**preliminary results were favorable; more testing soon**
Gelling time	+ / -	+ / -	+ / -	
Molten agarose consistency	+ / -	-	+	
Gel strength	-	- -	+	
Running time	+ / -	+ +	+	
Cost	- - -	- -	N / A	

N / A = not available. Information was not available for all chemical properties at the time of testing, and the price was not set for **New** Megabase agarose.
- = characteristic was relatively less than SeaKem Gold
+ = characteristic was relatively more than SeaKem Gold
+ / - = characteristic was similar to SeaKemGold

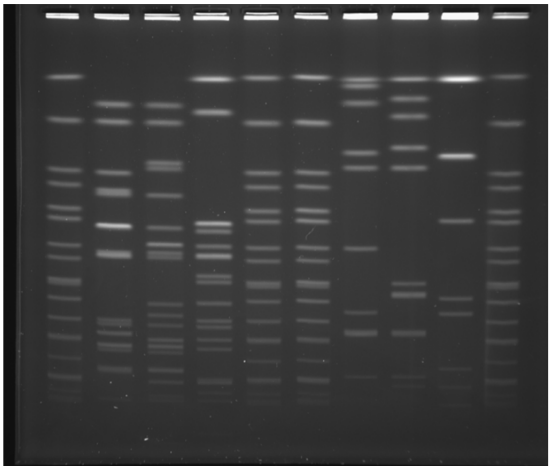
Figure 3. Comparison of *Salmonella enterica* PFGE patterns generated during external validation by PulseNet labs. Certification strains were digested with *Xba*I (panel A) or *Bln*I (panel B) and run by PFGE using standard conditions. Bands were marked and relationships analyzed within BioNumerics (Dice coefficient, UPGMA dendrogram, 1.5% optimization and 1.5% tolerance).



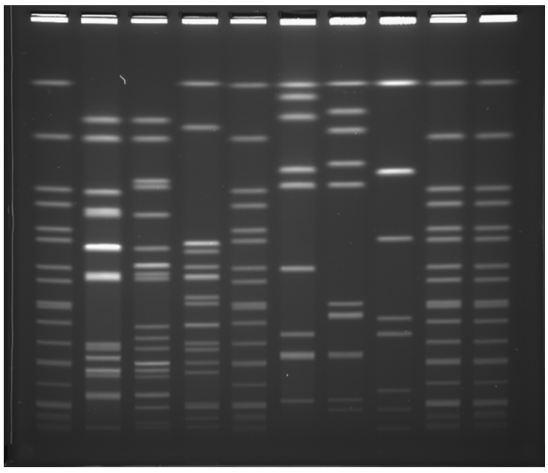
SeaKem Gold – Lonza



PFGE – IBI



"new" Megabase – Bio-Rad



LF™ – Amresco

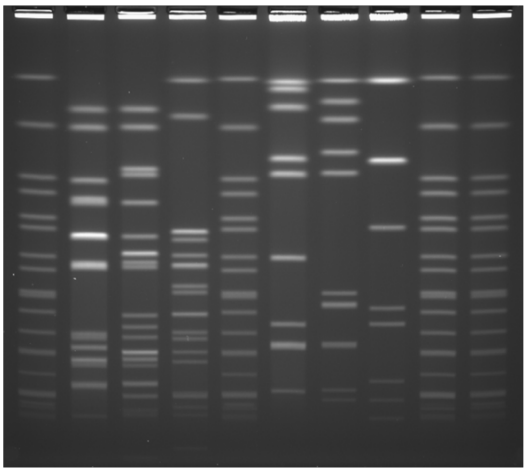


Figure 2. Comparison of patterns from routine isolates run on SeaKem Gold and three alternative agaroses. Each gel contains plugs made from the *Salmonella* certification strain set. All gels were run for 19 hours. Gels are courtesy of the Department of General Services, Division of Consolidated Laboratory Services in Virginia.

Summary

Overall, laboratories obtained a high level of gel and image quality regardless of which agarose was used. Some variability was noted between labs that could have been influenced by differences in TBE or water or technician-related but were within an acceptable range of variation for PFGE, based on gel normalization.

Recommendations

- **Run time:** Must be empirically determined and optimized in each lab. Run time could not be predicted, no obvious trends across labs or agarose brands and influenced by instrument, TBE, individual, etc…
  - Short gels often normalized poorly and resolution was also poor; improved normalization and resolution when gel length was good (last band within 1 cm of bottom of gel).
  - Adding run time not always the answer for poor normalization and some short gels may still normalize within an acceptable range.
  - Normalization is not adversely affected by either Amresco III™ or New Megabase. A few gap differences between large fragments were noted. These were most likely due to short run times and were within acceptable position tolerance for clustering.
- **Bio-Rad New Megabase**
  - acceptable for casting gels
  - acceptable for preparing plugs
  - may use with plugs cast with SeaKem Gold
  - Additional run time may be required (30 – 120 minutes) for optimum length; may not be compatible with lab workflow. Optimization is necessary prior to implementation.
- **Amresco LF (aka III™)**
  - acceptable for casting gels
  - **not** recommended for preparing plugs
  - may use with plugs cast with SeaKem Gold
  - run time may vary from lab-to-lab (more or less time or the same) – optimization is necessary prior to implementation
- **IBI Scientific PFGE Agarose**
  - currently being evaluated by CDC

- **Should my lab switch agaroses?**
  - **What to think about:** testing volume, simplicity, optimization, cost
  - Consider it if high volume and cost would be significant savings and your lab is willing to optimize run time and trouble shoot issues that may arise.

The PulseNet Reference Laboratory at the CDC would like to announce that laboratorians wishing to begin implementing these agaroses according to the recommendations above to run PulseNet PFGE gels are welcome to do so. The current PulseNet Standardized protocols will be edited to include these agaroses as options.